

BIOLOGY AND CONSERVATION OF OVERWINTERING MONARCH
BUTTERFLIES IN MEXICO

BY

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by

Alfonso Alonso-Mejía

To my parents from whom I received the
principles that I use in my life

To my brother and sister with whom I shared the joy of being a kid

To my wife from whom I receive constant love,
support, and understanding

To Eneida and Eduardo from whom I learned the art of dedication to the
conservation of monarch butterflies

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Abstract of Dissertation Presented to the Graduate School
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BIOLOGY AND CONSERVATION OF OVERWINTERING MONARCH
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By

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Chairman: Lincoln P. Brower
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Millions of monarch butterflies (Danaus plexippus) overwinter in México in forests dominated by the Oyamel fir tree (Abies religiosa). Logging practices and clearing for agricultural fields represent major threats to these forests. The Monarch Butterfly Special Biosphere Reserve (MBSBR) was created in 1986 to protect the monarch butterfly. The reserve experienced varying degrees of tree extractions before its creation such that monarchs currently form their overwintering aggregations in forests that contain both closed and opened areas. In an attempt to link ecological research with conservation strategies for the MBSBR, I investigated how forest microclimate and degree of disturbance affect the survival of monarch butterflies in the Sierra Chincua, one of the most pristine sites within the MBSBR. I defined closed and opened areas by using a new method that considered tree density, total basal area, and forest overstory density. Monarch butterflies that clustered in opened areas during the

1993-94 overwintering period experienced lower ambient temperatures during the night, higher wind velocities, higher rates of water evaporation, higher rates of lipid use, and higher rates of bird predation than monarchs clustered in closed areas. I detected high rates of bird predation possibly because monarch butterflies lose their chemical protection as they age. It appears that scale loss and denaturation are the most important factors explaining the decrease in cardiac glycoside concentration in adult monarch butterflies. Contrary to recent suggestions that logging is needed to provide more nectar sources for the monarchs, I found that the core area already has many artificial openings. In addition, the species composition of plants in flower is similar in artificially-opened and naturally closed areas. My research shows that there is no need to create additional artificial openings and that further thinning of the forest would be extremely harmful for the monarchs since it would increase the risk of mortality due to freezing, bird predation, and depletion of lipids needed for the monarchs' return migration to the southern United States. Future studies should address forest management plans and the improvement of current agricultural practices that would provide alternatives to logging for the local inhabitants.

CHAPTER 1 INTRODUCTION AND BACKGROUND

Monarch Butterfly Migration

The monarch has landed back in the land of the free and the brave. M. Zalucki wrote on e-mail 1995

Monarch butterflies (Danaus plexippus L.) are well-studied insects with amazing life history traits. Monarch caterpillars feed exclusively on milkweed plants (Asclepias spp.) from which they sequester chemical compounds that are toxic to several vertebrate predators when ingested (review in Brower 1984). The bright yellow and black colored stripes of the caterpillar and the orange and black colors of the adult serve to advertise the toxicity of the monarch. In addition to being one of the classical examples of aposematic coloration, monarchs are exceptional as the only insect species that performs long distance migrations over thousands of kilometers (Brower 1985, 1995a).

According to Kitching et al. (1993), the subgenus Danaus probably evolved in South America during the Pliocene from an Old World ancestor. After colonizing Central America, subsequent monarch ancestors reached North America and found a highly diverse milkweed flora (Woodson 1954). During the Pleistocene, the alternating glacials and interglacials caused contractions and expansions of the geographic ranges of both fir forest and milkweed flora that may have caused migratory movements in monarch

butterflies (Graham 1973; Brower 1985, 1995a). Migration and aggregation behaviors occur in other genera of the subfamily Danainae in the Old World (Danaus (Salatura), Euploea, Ideopsis, Parantica, and Tirumala), and in the New World (Anetia, Ivie et al. 1990; Wang and Emmel 1990; Scheermeyer 1993). Furthermore, the high numbers of monarchs that now migrate to México seem to be the result of large scale deforestation that occurred in eastern North America in the 1800's (Vane-Wright 1993). According to this hypothesis, the distribution and abundance of the milkweed plant Asclepias syriaca L. increased in newly cleared forest. The higher availability of food increased the density of monarch butterflies migrating to México.

Overwintering Biology

Each year during the autumn, the North American population of monarch butterflies east of the Rocky Mountains migrates to several mountain ranges in central México. As their larval food source of milkweed plants diminishes in North America at the end of the summer, monarchs escape the cold northern winter and migrate to the cool, moist environment of the Oyamel fir forest (Abies religiosa H. B. K.). Monarchs arrive in early November and form tightly packed aggregations of up to 10 million butterflies per hectare in several areas in the Transverse Neovolcanic Belt in the states of Michoacán and México (Urquhart and Urquhart 1978a, b; Brower 1985; Calvert and Brower 1986; Calvert et al. 1989; Calvert and Lawton 1993). There, they remain largely inactive, clustered on tree trunks and tree branches, and maintain a state of

reproductive diapause until March when they migrate back to the southern United States to exploit freshly emerging milkweed plants (Herman 1985; Brower and Malcolm 1991; Malcolm et al. 1993).

In the high altitude Oyamel fir forests, monarchs find cool ambient temperatures where they remain quiescent for most of the five months of the overwintering period (Brower and Calvert 1985; Calvert et al. 1989). This reduces the burning of the lipid reserves that are needed for their return migration to the southern United States (Chaplin and Wells 1982; Masters et al. 1988). Monarchs are periodically active when they are dislodged from tree clusters by predatory birds and winter storms, or when they fly out of the aggregation to drink water (Calvert and Cohen 1983; Calvert et al. 1983; Brower and Calvert 1985; Alonso-M. et al. 1993; Calvert 1994). Downslope colony movement to more humid areas also occurs as the dry season progresses (Calvert and Brower 1986; Calvert 1994). Lipid reserves remaining at the end of the overwintering period are used for migration and reproduction, and are probably supplemented by nectar feeding along the migration route to the southern United States (Heitzman 1962; Brower 1985; Urquhart 1987).

These high concentrations of monarchs are prime targets for several vertebrate predators. Monarch butterflies are a high quality resource for predators because low ambient temperatures make them largely inactive, and they possess large amounts of lipids (Brower 1985; Masters et al. 1988; Calvert et al. 1989; Malcolm and Brower 1989). Moreover, two- to six-month-old monarch butterflies overwintering in México are poorly protected chemically since the concentration of the chemicals that provide them protection decreases with age (Alonso-M. and Brower 1994). Thus, high rates of predation have been recorded at the overwintering sites in

México (Calvert et al. 1979; Brower and Calvert 1985; Glendinning et al. 1988; Arellano et al. 1993). Black-backed orioles (Icterus galbula abeillei Lesson) and black-headed grosbeaks (Pheucticus melanocephalus Swainson) consumed close to 9% of the Sierra Chincua monarch aggregation during the 1978-79 overwintering season (Brower and Calvert 1985). The scansorial black-eared mouse (Peromyscus melanotis J. A. Allen and Chapman) consumed close to 5% of an aggregation that formed at the same site in 1986 (Glendinning et al. 1988).

Most overwintering aggregations known to date form on the southwest facing slopes of the Mexican mountains (Calvert and Brower 1986). Southwestern slopes are usually wetter than northern and eastern slopes because moist rich air masses from the Pacific coast move into the mountains during the winter (Mosiño-Alemán and García 1974; Calvert et al. 1989). Monarchs also consistently form their aggregations at certain altitudes. At the beginning of the period, they almost never aggregate below 3,100 m. As the aggregations move to more humid areas at the middle-to-end of the overwintering period, they re-group at altitudes as low as 2,900 m (Calvert and Brower 1986). The overwintering period overlaps with the dry season which extends from November to April. The area receives more than 1,000 mm of rain during the summer wet season (Rzedowski 1983).

The Oyamel Fir Forest

When the axe came into the forest,
the trees said: the handle is one of us!
(On a bumper sticker, 1995)

Description

Most overwintering aggregations of monarch butterflies form in forests dominated by the Oyamel fir tree, an endemic species to the mountains of Central México (Rzedowski 1991). Oyamel fir forests are relicts of more extensive boreal-like forests which advanced during the glacial and interglacial periods of the Pleistocene (Graham 1973). Currently, these forests have island-like distributions on mountain peaks at elevations between 2,400 and 3,600 m where colder climates prevail and other tree species such as Pinus L. (Pinaceae), Quercus L. (Fagaceae), and Buddleia L. (Loganiaceae) occur at low densities (Loock 1947; Madrigal 1976; Manzanilla 1974; Rzedowski 1983). Cupressus lindleyi Klotzsch (Pinaceae) occurs in pure stands near the lower limits of the oyamel forest (Calvert et al. 1989; Soto and Vazquez 1993).

The understory vegetation consists primarily of herbaceous and bushy plant species in the Asteraceae (Senecio spp, Eupatorium spp, Stevia spp) and Lamiaceae (Salvia spp), with a diverse assortment of ascomycetes, basidiomycetes and bryophytes (Espejo et al. 1992). Ground cover includes Acaene elongata L., Alchemilla procumbens Rose (Rosaceae), and in some areas a carpet of mosses including species in the genera Thuidium and Mnium (Calvert et al. 1986). High altitude meadows (i.e. llanos) occur in some flat areas where the drainage is restricted, the soils freeze, and the vegetation is dominated by grasses (Potentilla candicans H. & B., Rosaceae)

and forbs. Llanos are usually bordered by the bush-sized Juniperus monticola var. compacta Martinez (Pinaceae) and by Baccharis conferta H. B. K. (Asteraceae, Snook 1993; Soto and Vazquez 1993). Attempts to reforest these llanos with fir trees have failed, and should not be encouraged since the habitat is not appropriate for tree survival (Snook 1993).

Oyamel trees have small needle-like leaves. This facilitates the ability of monarchs to cluster close to one another. Moreover, the architecture of fir trees allows them to support heavy loads of ice and snow (Heinrich 1996) such that the branches of the Oyamel fir trees support large numbers of monarch butterflies without breaking (Alonso-M. 1996).

Unfortunately, the ecology of the Oyamel fir tree has not been studied in detail. For example, little is known about the appropriate field conditions for seed germination and seedling establishment and survival. It is known that fir trees can be infected by bark beetles (Scolytidae: Scolytus hermosus, S. mundus, S. ventralis, Pityophthorus blackmanii, and Pseudohylesinus variegatus, Hernández and Cibrián 1981), mistletoes (Arceuthobium abietis-religiosae, Rodríguez 1983), and periodic outbreaks of geometric moths (Evita hyalinaria blandaria Dyar Geometridae, Carbajal and López 1987), and that any of these infections can lead to tree mortality (Snook 1993). However, further studies on the distribution, abundance, and vertical and horizontal transmission of these diseases are needed.

Most Oyamel trees die standing by parasitism or by lightning. Thus, small scale disturbances such as tree fall gaps are not observed in the area. During the snow storm of 1981, Calvert et al. (1983) reported that one tree was uprooted but such events are rare in the forest. Little is known of the effects (positive or negative) of forest fires in Oyamel trees. According to Gutiérrez (1983) most forest fires are set intentionally by cattlemen and

farmers to promote new spring grass growth for their animals and to clear forested areas for planting as the agricultural frontier expands up the mountains. The conversion of forest land to agricultural use is a key factor in forest destruction, leading to soil erosion and poor productivity (Snook 1993).

Forest Degradation

Oyamel trees are extracted from the forest for commercial, industrial and domestic purposes (Snook 1993). Commercial exploitation provides raw materials for local sawmills and neighboring conglomerate board and papermaking industries. Legal exploitations require the issuing of permits for extraction and transportation of authorized volumes of wood and they are carried out in accordance with the Mexican Method of Forest Control (Método Mexicano de Ordenación de Montes, Musalem 1979). This method of forest management involves a low-intensity, periodic selective cutting of 35-40% of the volume of the desired tree species. It attempts to increase the growth of the remaining trees, enhancing production, and regeneration by reforestation. However, lumbering operations in the Oyamel forests have not shown the expected results, since the area is not large enough to allow a rotation that will permit forest regeneration. Moreover, logging temporarily destroys large areas of the understory vegetation where trees are felled and logs are transported to the trucks. The method also requires reforestation practices. However, free-ranging livestock are commonly found in the area and seem to have a negative effect on the survival of oyamel fir tree seedlings (Calvert et al. 1989; Snook 1993).

According to Snook (1993), the uncontrolled timber cutting for domestic purposes can have an even higher negative effect on the

degradation of the forest. She argues that significant quantities of wood are extracted for construction materials (beams and shingles "tejamanil") and fuelwood (for cooking and heating purposes) by the large populations of local peasants that live in the surrounding areas near the overwintering sites (i.e. about 15 000 people live in the Sierra Chincua, Campanario, and Chivati-Huacal region). She estimated that approximately 75,000 m³/yr, or about 40,000 fir trees of average size (1-3 m³), are taken each year. Most of the local peasant population lives at a subsistence level deriving their food from agriculture and grazing, their fuel and construction materials from the forest, and their income from the sale of agricultural goods and wood products (Chapela and Barkin 1995). The amount, origin and destination of wood taken by each ejido for domestic purposes needs to be evaluated. The rate of population growth of these communities also needs to be studied and considered in future forest planning. Emigration to Mexico city seems to be high but needs to be quantified (Chapela and Barkin 1995).

In addition to logging, local inhabitants obtain a limited number of non-timber products from the oyamel forests. These include the collection of flowers for religious rituals, herb plants for medicinal purposes (e.g. "te de monte" from Satureja macrostema, Lamiaceae), the extraction of resin from pine trees, and the harvesting of mushrooms during the rainy season.

The restricted distribution of the Oyamel forest to high altitude mountain peaks, and the increasing pressure from logging and clearing for agricultural fields make it more vulnerable to deforestation than any other forest type in México (Calvert et al. 1989; Snook 1993). The degradation of the forest endangers the migratory phenomenon of the monarch butterfly because the method of selective cutting of trees to maximize timber production reduces the density and canopy coverage of the

forest needed to insulate the butterflies against extreme cold temperatures that occasional winter storms bring to the area (Calvert et al. 1983; Wells et al. 1983; Brower and Malcolm 1991; Culotta 1992; Anderson and Brower 1996; E. Rendón unpublished data). Thus, a reserve was created to protect the forest of five areas from logging where monarchs overwinter.

The Monarch Butterfly Special Biosphere Reserve

The overwintering sites in México were first discovered by F. A. Urquhart and colleagues after several decades of research on monarch migration (Urquhart 1976; Urquhart and Urquhart 1976). Concerned about the preservation of the spectacular migratory phenomenon, the Urquharts decided not to share the exact location of their findings (Brower 1995a). Despite this, the publication of popular and scientific articles on monarchs overwintering in México soon made the sites known (Urquhart 1976; Brower 1977, 1985; Barthelemy 1978; Urquhart and Urquhart 1978a, b; Calvert et al. 1979; Calvert and Brower 1986; review in Brower 1995a).

W. Calvert and L. Brower, from the University of Florida, conducted pioneering research on the biology of monarch butterflies overwintering in México. In collaboration with Leonila Vazquez and Hector Perez, professors of entomology at the National University of México, Calvert and Brower soon learned that the Oyamel fir trees were being commercially exploited at a fast rate. They determined that the survival of overwintering monarchs was closely related to the microclimate registered in closed canopy forests, such that creating opened areas by logging enhanced

monarch mortality (Calvert and Brower 1981; Calvert and Cohen 1983; Calvert et al. 1982, 1983).

It was not until 1986 that five forested mountain tops in the States of Michoacán (Cerro Altamirano, Sierra Chincua, Sierra Campanario, and Cerros Chivati-Huacal) and México (Cerro Pelón) were designated as protected through the creation of the Monarch Butterfly Special Biosphere Reserve (MBSBR) by the signing of a decree by Mexican President Miguel de la Madrid (Diario Oficial 1986). Further investigations by Calvert determined that monarchs form an additional four to seven overwintering areas depending on the year (Calvert and Lawton 1993). These include Cerro San Andrés, Mil Cumbres, Cerro Picacho in the State of Michoacán, and Cerro las Palomas, Oxtotilpan, Cerro Piedras Chinas, and Piedra Herrada, in the State of México. Most of these overwintering aggregations form along an arc stretching from the western slopes of Volcano Nevado de Toluca in the state of México, northwest to the city of Zitácuaro and north to the Altamirano mountain in the state of Michoacán (Figure 2 in Calvert and Brower 1986; Calvert et al. 1989; Calvert and Lawton 1993). The total length of the arc from Palomas, the most easterly site, to Altamirano, the most northerly aggregation, is approximately 150 km.

The MBSBR was classified as special because of the relative small area that it protects (Halfpeter 1984). It includes 16,110 ha of which 11,600 ha are classified as buffer zones where forest extractions are permitted (Diario Oficial 1986; Table 1.1). Therefore, logging-free wilderness areas consist only of 4,500 ha. Core areas were created for protection of the animal and plant species found in the relict Oyamel fir forests, and to serve as sources of species to recolonize logged areas in the buffer zones. In the Chincua area, 700 ha were purchased by the federal government and 80 ha were

expropriated by the State of Michoacán for protection. The remaining land, nearly 15,500 ha, is communally owned land (called ejidos) granted to more than 30 groups of organized peasants. Before 1994, when President Carlos Salinas modified the 27th article of the Mexican Constitution (Diario Oficial 1994), ejidos could not be bought, sold or transferred. With the new law, ejidos can be divided and each ejidatario (owner), can sell his part. However, the decision to divide an ejido has to be accepted by the majority of the ejidatarios. Ejidos affected by the MBSBR have kept the same organization as if the law had not been changed. They democratically select a leader (comisario) and a treasurer every two years. Each can be re-elected only once.

The different areas of the MBSBR experienced varying degrees of tree extractions before the creation of the reserve such that monarchs currently form their overwintering aggregations in forests that contain both opened and closed areas. Calvert et al. (1989) found the tree density in the overwintering sites ranges from 90 to 620 trees/ha and the basal areas varies from 12.1 to 43.8 m²/ha. They compared these data to previous studies on Oyamel forests in México (Madrigal 1967; Manzanilla 1974), and to forest stands of Abies amabilis (Dougl.) Forbes in the Cascade Mountains of the northwest, and A. balsamea (L.) Mill. in the Adirondack Mountains from the northeastern United States (Grier et al. 1981; Sprugel 1984). They found that the forest in these previous studies had higher densities in younger and mature stands, and higher, age-related basal areas than the Mexican fir forests. They concluded that with the possible exception of the Chincua and Herrada overwintering sites, the Oyamel fir forest stands studied have being heavily exploited.

Core areas of the monarch reserve are already subject to numerous human activities. Out of the 4,500 logging-free hectares of the reserve, 1,000 are being used as centers for ecotourism, one of the major alternative incomes to ejidatarios (Campanario and Pelón). Fifteen hundred hectares have been illegally logged and the remaining 2,000 ha have a mosaic of closed and open forest patches where most of the scientific research on the migrating monarchs and on the oyamel forest is conducted (A. Alonso-M. unpublished data). Since the core areas the MBSBR are small, for them to serve as sources of plant and animal species for recolonization of the 11,600 ha of the buffer zone, they should be increased in size and maintained as logging-free areas. Ideally, unprotected overwintering aggregations should be incorporated into the MBSBR so that the risk of extinction of the endangered phenomenon of the monarch butterfly migration will decrease.

This Study

In an attempt to link ecological research with conservation strategies for the MBSBR, I investigated how forest microclimate and degree of disturbance affect the survival of monarch butterflies in the Sierra Chincua, one of the most pristine overwintering sites in México (Calvert et al. 1989; Calvert and Lawton 1993). I first designed an objective and practical method to determine closed and open areas in these forests. I then monitored daily changes in temperature, humidity, and wind speed in closed and opened areas during most of the 1993-1994 overwintering season. I also experimentally compared the rates of water evaporation and the rate of lipid consumption for monarchs that were experimentally exposed to

closed and opened climatic conditions (Chapter 2). I found that monarchs in opened areas experienced lower ambient temperatures during the night, higher ambient temperatures during the day, higher wind velocities, and higher rates of water evaporation. This indicates that butterflies clustering in opened areas have a higher risk of freezing mortality, higher rates of dehydration, and higher rates of lipid use. The higher rate of lipid loss in monarchs clustered in opened areas is consistent with the hypothesis that intact, closed forest is necessary for successful overwintering because this permits them to conserve their lipid reserves for the spring migration back to the United States. In fact, the San Andres overwintering monarch aggregation that forms outside the limits of the MBSBR protected area (map in Calvert and Brower, 1986), and the highly disturbed Altamirano and Chivati-Huacal monarch aggregations have become smaller, ephemeral, unstable and in several years have failed to form (W. H. Calvert, unpublished data). These changes seem to be in response to low tree densities resulting from current and past forest extractions.

I followed how inactive monarchs clustered on trees utilized their lipid reserves throughout the overwintering period. I compared their rate of lipid use to monarchs that were collected while visiting flowers in the surroundings of the overwintering aggregation. I also compared lipid amounts in autumn migrants collected in Texas, spring migrants collected in the southern United States, and non-migratory, reproductively active summer generations collected in Wisconsin and Minnesota (Chapter 3). I found that clustered butterflies had significantly higher lipid mass, water content, lean mass, and larger wings than did monarchs collected from flowers. These differences were consistent throughout the overwintering period. A high proportion of flower-visiting monarchs had lipid mass close

to zero, and very few of the butterflies had medium to high lipid levels. This data suggests that flower-visiting monarchs may be a derived group from the clustered monarchs and may represent a continual recruitment of those individuals that are approaching starvation.

Despite the mounting evidence indicating that logging is detrimental to the survival of overwintering monarchs (Calvert and Brower 1981; Calvert et al. 1982, 1986, 1989; Calvert and Cohen 1983; Brower and Malcolm 1991; Alonso-M. et al. 1992; Snook 1993; Anderson and Brower 1996), Hoth (1993) and Chapela and Barkin (1995) argue that logging should be permitted in the core areas of the reserve. Their argument to justify logging is that tree extraction would benefit monarchs by creating forest openings in which more plants would flower. This reasoning maintains that the increased availability of nectar could translate into fewer monarchs depleting their lipid contents, and therefore, more monarchs surviving the overwintering period. In Chapter 4, I looked at the frequency of opened and closed areas and the distribution of flowering plants in both closed and opened areas. I found that the core area of the Sierra Chincua MBSBR already has a substantial number of areas with human-induced disturbances, that the plants in flower were common during the winter at this site, and that natural and artificially-opened areas had the same plant species composition.

In order to investigate how increased logging may impact monarch survival in the MBSBR, I also studied how monarch butterfly mortality caused by bird predation is currently affected by logging extractions that occurred in the early 1980s (Chapter 5). I monitored daily bird predation in closed and opened areas and found that monarch butterflies overwintering

in areas with low tree density, low basal area, and low canopy coverage had higher rates of bird predation than monarchs in areas with closed forest.

I also investigated three mechanisms by which adult monarchs may lose their toxic cardiac glycoside as they age, since this may help explain the high rates of bird predation observed at the overwintering sites (Chapter 6). Based on the data from a series of experiments, it appears that scale loss and denaturation of the toxic compounds are the most important factors explaining cardiac glycoside decrease in adult monarch butterflies. The observed decrease in cardiac glycoside concentration results in a lower level of chemical defense for adult monarch butterflies as they age. Since monarchs are more active in opened areas, they may age and lose scales faster than those in closed areas.

These new data provide further evidence against issuing logging permits in the core areas of the MBSBR because logging opens the forest and an opened forest is detrimental for monarch survival. A very important aspect of the conservation efforts relates to the socioeconomic situation of the local inhabitants. In Chapter 7, I discussed alternative sources of forest and agricultural management that could provide alternatives sources of income to the local people.

Table 1.1. Total area for the core and buffer zones for each of the five protected overwintering sites of the Monarch Butterfly Biosphere Reserve. Data from Diario Oficial (1986).

	<u>CORE ZONE</u>	<u>BUFFER ZONE</u>	<u>TOTAL</u>
Altamirano	244	1133	1377
Chincua	1060	1635	2695
Campanario	900	988	1888
Chivati-Huacal	940	1074	2014
Pelon	687	6787	7474
TOTAL	4490 ha	11619 ha	16110 ha

CHAPTER 2

MICROCLIMATIC DIFFERENCES BETWEEN CLOSED AND OPENED FOREST AND THEIR CONSEQUENCES FOR THE SURVIVAL OF MONARCH BUTTERFLIES OVERWINTERING IN MEXICO

Introduction

Microclimate is a major factor in the survival of monarch butterflies (Danaus plexippus L.) overwintering in México. Every year, monarchs migrate from eastern North America to high altitude, cool, humid Oyamel fir (Abies religiosa H. B. K.) forests in the mountains of central México (Mosíño-Alemán and García 1974; Brower 1985; Calvert and Brower 1986; Calvert et al. 1989). Cool ambient temperatures benefit monarchs in at least two ways. Since temperatures during the day are usually below flight threshold (Masters et al. 1988; Alonso-M. et al. 1993), the majority of butterflies remain quiescent for most of the five month overwintering period. This reduces burning of their lipid reserves, which are needed for the return migration to the southern United States (Chaplin and Wells 1982; Masters et al. 1988). Monarchs are periodically active during their overwintering period when dislodged from tree clusters by predatory birds and winter storms and when they fly out of the aggregation to drink water (Calvert and Cohen 1983; Calvert et al. 1983; Brower and Calvert 1985; Alonso-M. et al. 1993; Calvert 1994). Furthermore, at the low temperatures prevailing in the overwintering aggregations, monarchs remain in a physiological state of reproductive diapause that represses the maturation

of their gonads, controlling lipid utilization and aging (Barker and Herman 1976; Dallman and Herman 1978; Lessman and Herman 1983; Herman 1985; Herman et al. 1989).

In 1986, five limited areas of the Oyamel fir forest where monarchs overwinter were designated as protected through the creation of the Monarch Butterfly Special Biosphere Reserve (MBSBR; Diario Oficial 1986). These protected areas had experienced different degrees of tree extraction before the creation of the reserve so that monarchs that form their overwintering aggregations there do so in both closed and opened areas of forest. Calvert et al. (1982) reported that colder temperatures during the night were registered in areas that had been thinned by logging, and suggested that monarchs perched in opened areas would have higher risks of freezing mortality. Overwintering monarchs have the capacity to acclimate rapidly to low ambient temperatures (i.e. cold-hardening) and to survive temperatures moderately below freezing. However, when the ambient temperature drops below the subzero temperature at which spontaneous tissue freezing occurs (i.e. the supercooling point), the butterflies die since they are freeze-susceptible insects (Salt 1961; Lee 1989; Lee et al. 1987; Alonso-M. et al 1992; Larsen and Lee 1994; Anderson and Brower 1996).

During a snow storm in January 1981, Calvert et al. (1983) found that 42% of a monarch aggregation perished in the Sierra Chincua, Michoacán (see map in Calvert and Brower 1986). In 1992, Brower (in Culotta 1992) reported that 83% of the Herrada overwintering aggregation in the State of México were killed by freezing. In Michoacán, 6% of the Mojonera Alta aggregation perished at the end of 1995 after a snow storm hit the mountains (E. Rendón unpublished data). On these occasions, monarchs

that remained dry survived ambient temperatures as low as -8°C . Wet monarchs, however, died at ambient temperatures of -4°C , when ice crystals formed on their body and triggered internal ice nucleation via their spiracles (Salt 1961; Alonso-M. et al. 1992; Larsen and Lee 1994; Anderson and Brower 1993, 1996). Anderson and Brower (1996) have recently shown that monarchs perched in areas of closed forest and those inside and on the bottom of bough clusters are better protected from accumulating water on the surface of their bodies than are those on the outside of a cluster and those perched in opened areas. Monarchs may select perching sites within the overwintering aggregation where they can remain dry.

In this chapter, I extended the study by Calvert et al. (1982) by closely monitoring microclimatic conditions in well-defined closed and opened areas within a monarch aggregation over the entire overwintering period. I first used an objective method to locate closed and opened areas within the forest. I then monitored and compared daily changes in temperature, humidity, and wind speed in these areas during most of the overwintering period. I hypothesized that closed areas would be more buffered (i.e. narrow range in variation) than opened areas.

The overwintering period of monarch butterflies overlaps with the dry season of the area (November to April, Rzedowski 1983; Calvert et al. 1989). Under such dry conditions, it may be important for monarchs to select perches that minimize body water loss. I designed an experiment to test if evaporation was higher in opened than in closed areas. In a second experiment, I tested if monarchs clustered in opened areas consumed their lipid reserves at higher rates than those in closed areas. I predicted that since monarchs perched in opened areas would be exposed to more sunlight, they could experience higher body temperatures and exhibit a

higher rate of lipid consumption (Chaplin and Wells 1982; Masters et al. 1988). Data gathered in this study will help us better understand the relation between the structure of the oyamel fir forest and the biological needs of monarch butterflies overwintering in México.

Methods

Study Site

This study was conducted at the Llano del Toro overwintering monarch aggregation, located on the southwestern facing slope of Zapatero Canyon at the Sierra Chincua, a mountain range in the Transverse Neovolcanic Belt in northeastern Michoacán (19°40'48"N and 100°17'54"W, Anonymous 1976). This overwintering aggregation has formed almost every year since the overwintering sites were discovered in 1975 (Urquhart and Urquhart 1976; Calvert and Brower 1986; Calvert et al. 1989).

Microclimatic Conditions in Closed and Opened Areas

I used on-site weather loggers (OWL, Electronically Monitored Ecosystems, 2229 Fifth St., Berkeley, CA 94710) to record ambient temperature (°C), relative humidity (%), and wind velocity (m/sec) from December 12, 1993 to March 24, 1994. OWLs were programmed to record measurements every minute and to save the average of those measurements over two hr periods. I had four recording stations, two in closed and two in opened areas. Each recording station consisted of three temperature probes, three relative humidity probes, and three anemometers. All probes were placed near clustered butterflies at three

meters above the ground. One of the stations in a closed area did not have an anemometer.

I determined if an area was closed or opened based on a Closed/Opened Index. Six parallel transects were established and each was subdivided into 14 contiguous 10 X 10 m quadrats. Tree density (number of trees/quadrat), total basal area (based on the diameter at breast height for all trees found per quadrat), and forest overstory density (based on spherical densiometer readings, Lemmon 1957) were estimated for each quadrat. Using data from all quadrats, I obtained a relative value based on the maximum datum recorded for each variable. The three relative values estimated for each quadrat were added to obtain a quadrat-index value. The average of all quadrat-index values was used to obtain a Closed/Opened Index (average = 56.9, S.E. = 1.39, range = 32.7-97.0, $n = 75$). Quadrats with higher quadrat-index values than the Closed/Opened Index were classified as closed, while quadrats with lower quadrat-index values than the Closed/Opened Index were classified as opened. Using this method, I classified 39 of the quadrats as closed and 36 as opened. Nine of the 84 quadrats were excluded from the analysis because they occurred on wide hiking trails or abandoned logging roads. I randomly selected two closed (Closed/Opened Indices = 85.5 and 71.2) and two opened quadrats (Closed/Opened Indices = 50.3 and 49.3) in which to set the recording stations.

Experiment 1. Estimates of Water Evaporation

I compared rates of water evaporation in closed and opened areas by estimating the amount of distilled water that evaporated from plastic tubes (8.5 cm height X 3 cm diameter). I put 40 ml of distilled water in each tube

and sealed it with a one-hole rubber stopper. A dental cotton wick (15 cm long X 1 cm diameter) extended from the bottom of the tube, to about four cm above the rubber stopper. A small plastic net covered the cotton to prevent insects from drinking the water. The tubes were hung three meters above the ground, in the shade, near clustering monarchs. I set one tube in each of 10 closed quadrats and 10 opened quadrats. The rate of water evaporation (in ml/week) was measured from 20 December, 1993, to 24 March, 1994 ($n = 13$ weeks).

Experiment 2. Monarch Lipid Consumption in Closed and Opened Areas

I performed an experiment with netted butterflies to determine if monarchs had higher rates of lipid use when they were exposed to conditions of opened areas. On February 2, 1986, I collected 500 monarchs from three clusters that were three to four meters above the ground. I estimated the wet mass of each butterfly to the nearest milligram using a Sartorius 1205 MP electronic balance and selected 240 butterflies that weighed between 500 - 650 mg. Forty butterflies (20 females and 20 males) were collected as controls and were frozen the same day I started the experiment for subsequent lipid analysis. Fifty butterflies (25 females and 25 males) were placed in each of four cylindrical nets (120 cm length X 30 cm diameter). Two nets were placed in closed and two in opened areas and hung two and a half meters above the ground. Here, opened areas consisted of sites that had no standing trees within a 200 m² area (approx. 15 X 15 m), while the closed areas had standing trees that prevented sunlight from reaching the understory vegetation.

All butterflies were fed water every other day by extending their probosces into a wet sponge. On February 22, I removed five of each sex

from each net ($n = 40$) and froze them. On March 8, I removed and froze eight females and seven males from each net ($n = 60$). I ended the experiment on March 23 when I collected and froze all individuals that were still alive (32 females and 21 males). Monarchs were placed in individual glassine envelopes and stored in a freezer at -4°C until lipid analysis was performed. I recorded ambient temperatures in both areas with Max-Min Taylor thermometers. Maximum ambient temperatures in closed areas ranged from $8 - 17^{\circ}\text{C}$, while in opened areas they ranged from $10 - 25^{\circ}\text{C}$.

I extracted lipids using the method of Walford (1980) and May (1992). Each monarch was dried at 60°C for 16 hr, weighed, and ground in a centrifuge tube in 20 ml petroleum ether with a Janke & Kunkel SDT Ultra Turrax tissuemizer. Each sample was then placed in a medium speed shaker bath at 35°C for 30 min, with vortexing every 10 min. Tubes were centrifuged in a Dynac Clay Adams centrifuge at 1000 RPM for seven minutes, and the supernatant was decanted into preweighed aluminum pans on a 30°C hot plate. I added 15 ml of ether to the remaining solids in the centrifuge tube, and repeated the procedure described above. The weighing pans with the supernatant were allowed to evaporate overnight. I then weighed the remaining residue (mg), which was composed mainly of lipids.

Statistical Analyses

I compared the slopes and the y-intercepts of the regression lines of the sampling date (covariant) against the minimum ambient temperatures (response variable) registered in closed and opened quadrats (nominal variables) by using analysis of covariance (ANCOVA; Zar 1984). I used a

similar analysis to test the effects of the maximum ambient temperatures, the minimum and maximum relative humidities (arcsin transformed), and the maximum wind velocity. I used a repeated measures ANOVA to test if water evaporation was higher in opened areas. I used two-way ANOVA (treatment X date) to compare monarch lipid consumption in closed and opened areas.

Results

Microclimatic Conditions in Closed and Opened Areas

As the overwintering season progressed, ambient temperatures increased in both closed and opened areas. The lowest temperatures occurred at 0600 - 0800 hr, and maximum temperatures at 1600 - 1800 hr in both areas. Minimum temperatures registered in opened areas were consistently lower during the night than those in closed areas (Table 2.1; Fig. 2.1). The average daily minimum temperature registered in opened areas was 3.8°C (S.E. = 0.14, n = 96), compared to 4.9°C in closed areas (S.E. = 0.14, n = 103). Contrary to my expectations, the average daily maximum temperature of 12.9°C (S.E. = 0.26, n = 95) in opened areas did not differ from that in closed areas (average = 12.3°C, S.E. = 0.22, n = 103). Likewise, average daily minimum relative humidity (opened: 55.7%, S.E. = 1.25, n = 75; closed: 55.6%, S.E. = 1.44, n = 83), and maximum relative humidity (opened: 84.3%, S.E. = 0.81, n = 75; closed: 83.4%, S.E. = 1.02, n = 83) did not differ significantly between closed and opened areas ($p > 0.05$). Relative humidities remained high during most of the overwintering period (Fig. 2.2). I did not find correlations between minimum or maximum

temperatures and minimum or maximum relative humidities (Coefficients of determination, r^2 , $p > 0.05$).

Wind velocity increased significantly throughout the overwintering period in both closed and opened areas (Fig. 2.3). In closed areas, wind velocity increased from an average maximum of 0.91 m/s (S.E. = 0.06, $n = 20$ days) at the beginning of the season to 2.01 m/s (S.E. = 0.12, $n = 20$) at the end. Opened areas increased from 1.76 m/s (S.E. = 0.14, $n = 20$) to 2.60 m/s (S.E. = 0.07, $n = 20$). As expected, wind speed was consistently higher in opened areas throughout the season (Fig. 2.3; Table 2.1). During most of the season, wind was consistently stronger between 1200 - 1800 hr in both closed and opened areas (Fig. 2.4).

Experiment 1. Estimates of Water Evaporation

The rate of water evaporation significantly increased as the overwintering season progressed both in closed (ANOVA $F(1, 128) = 16.5$, $p < 0.001$) and opened areas (ANOVA $F(1, 128) = 68.9$, $p < 0.001$; Fig. 2.5). However, the overall rate of water loss was significantly higher in opened areas (Repeated Measures ANOVA $F(1, 216) = 5.99$, $p < 0.025$). There was high variability in the amount of water that evaporated each week in both closed and opened areas. In opened areas, an average of 24.7 ml of distilled water evaporated per week (S.E. = 0.72, $n = 130$), while 21.9 ml evaporated per week in closed areas (S.E. = 0.66, $n = 130$).

Experiment 2. Monarch Lipid Consumption in Closed and Opened Areas

At the beginning of the experiment, monarchs had an average lipid mass of 68 mg (S.E. = 5.3, $n = 40$). Using a two-way ANOVA, I found that both treatment (ANOVA $F(1, 145) = 37.2$, $p < 0.001$) and date ($F(1, 145) = 11.8$,

$p < 0.001$) had a significant effect on lipid content. I used the Ryan's Q test for multiple comparisons (Day and Quinn 1989) to compare the average lipid mass of butterflies in opened and closed areas on each date. I found that by March 8 and March 23, monarchs held in closed areas had significantly higher lipid contents than monarchs held in opened areas (Fig. 2.6). After six weeks, monarchs in opened areas had lost an average of 56.8 mg (S.E. = 2.3, $n = 26$, 83.5%) of their initial lipid weight, while monarchs in closed areas only lost 27.6 mg (S.E. = 5.0, $n = 27$, 40.6%).

Discussion

In this study, monarch butterflies overwintering in closed areas within the Oyamel fir forest at the Sierra Chincua found cool ambient temperatures, high relative humidities, and low wind velocities. These microclimatic conditions helped monarchs to remain quiescent for most of the overwintering period, maintaining low rates of lipid and water loss. Monarchs clustered in opened areas within the forest, however, experienced lower ambient temperatures during the night and higher wind intensities during the day. I also recorded higher rates of water evaporation in opened areas. Thus, monarchs clustered in opened sites had a higher risk of freezing mortality (Calvert et al 1983, Anderson and Brower 1996), they could have had higher rates of water loss, and were likely to deplete their lipid reserves before March, when they need them to migrate back to the United States. Monarchs may seek perching sites in areas of closed forest with better climatic conditions. In México, about 10 million monarch butterflies aggregate in less than 500 trees per hectare

(Calvert and Brower 1986, see chapter 5). At such high densities, preferred sites may be filled quickly, forcing many monarchs to perch in more opened areas. By enhancing Oyamel fir seedling regeneration and the preventing of illegal logging, opened areas would eventually become more closed, providing preferable microclimatic conditions and reducing monarch mortality.

Lower temperatures were registered in opened areas during the night than in closed areas. In general, objects cool at night because they lose heat by radiation to the atmosphere and by convection to surrounding air and other objects (Geiger 1965; Calvert and Brower 1981; Calvert et al. 1982, 1986; Anderson and Brower 1996). In a closed forest, the radiation, absorption, and re-radiation of heat from ground to foliage, foliage to foliage, and foliage to ground reduce the amount of heat lost to the atmosphere, and result in warmer temperatures. In contrast, opened areas have fewer plants from which heat can be re-radiated and more gaps through which infrared radiation escapes to the open sky. During cloudy or foggy nights, the water present in the atmosphere absorbs and radiates heat back to the emitting objects, retarding radiation loss to the night sky, and thus maintaining similar ambient temperatures in closed and opened areas (Geiger 1965; Calvert et al. 1982). Overwintering monarch butterflies cluster on the middle and lower portions of the trees and avoid the canopy where, in a closed forest, most of the radiational cooling occurs. Monarchs thus may cluster very close to each other not only to reduce bird predation (Brower and Calvert 1985; Arellano et al. 1993; Chapter 5), or to reduce the risk of getting wet during a storm (Anderson and Brower 1996), but also to reduce heat loss during the night.

Previous field studies on the cryobiology of overwintering monarchs butterflies in México have used naturally opened areas (i.e. meadows) where high rates of radiational cooling occur and where unusually cold temperatures have been recorded (Calvert and Brower 1981; Calvert and Cohen 1983; Calvert et al. 1986; Alonso-M. et al. 1992). However, during the 1981 snow storm, Calvert et al. (1983) registered average ambient temperatures of -4.1°C , with readings as low as -5.0°C at several locations within the Oyamel fir forest. Since many monarchs became wet during the rainy period prior to the snow storm, their supercooling capacity was greatly reduced. Their supercooling point was raised to -4°C from -8°C (the temperature at which spontaneous tissue freezing occurs). This caused an estimated mortality of 2.5 million monarchs. Calvert et al. (1982) also showed that thinned logged areas were 1.19°C colder than areas of closed forest (compared to a 1.18°C temperature difference in my study). I propose that during the 1981 and other snow storms (Calvert et al. 1983; Culotta 1992; E. Rendón-S. unpublished data), monarchs clustered in closed areas were better protected against freezing mortality than monarchs clustered in opened areas. I hypothesize that a single, warmer degree in the ambient temperature, and the reduced accumulation of water on the body of monarchs clustered in closed areas (Anderson and Brower 1996), could greatly decrease the susceptibility of monarchs to death by freezing.

Maximum ambient temperatures recorded in the shade did not differ between closed and opened areas (Fig. 2.1). I found, however, that after six weeks of experimentally holding monarch butterflies in opened areas, they consumed 83.5% of their initial amount of lipids. Monarchs held in closed areas only consumed 40.6% (Fig. 2.6). Adult monarchs are well suited to gain heat such that they can rapidly bring up their thoracic temperature

well above the ambient (Masters et al. 1988). Their relatively large body size, their coat of dark hair-like scales on the thorax, and the large wing mass near the thorax facilitates rapid heating (Church 1960; Douglas 1978; Kingsolver and Koehl 1985; Masters et al. 1988; Masters 1993). Douglas (1978) showed that monarchs in resting position (i.e. with their wings closed) increased their body temperature 10°C above the ambient when exposed to a source of light in the laboratory. Thus, the morphological characteristics that usually favor activity at cooler temperatures caused monarchs perched in opened areas to increase their body temperature and consequently increase their rate of lipid use (Chaplin and Wells 1982; Masters et al. 1988; Masters 1993). These results agree with published observations by Leong (1990), who found that overwintering monarchs in California avoid trees that have direct sun exposure and bright illumination.

Results from this study strongly suggest that wind velocity is an important environmental factor for monarch butterflies, as has been suggested for monarchs overwintering in California (Leong 1990; Leong et al. 1991). I found that monarchs clustered in opened areas were consistently exposed to stronger winds than monarchs in closed areas (Fig. 2.3). In addition, the highest wind velocities were recorded at the time of maximum dryness during the day (Fig. 2.4). Thus, the faster movement of dry air in the opened areas may explain the faster rates of water evaporation that I recorded in the evaporation experiment (Fig. 2.5). Stronger winds in opened areas may have also caused monarchs to be dislodged from clusters more frequently than in closed areas. I have found significantly higher numbers of live butterflies underneath clusters in opened areas compared to closed areas (see Chapter 5).

In spite of the prevailing dryness during the winter months in Central México (Rzedowski 1983; Calvert et al. 1989), data gathered in this study showed that relative humidities remained high at the Sierra Chincua overwintering site (Fig. 2.2). Monarch butterflies form their overwintering aggregations primarily on the southwest facing slopes of the Mexican mountains (Calvert and Brower 1986). Southwestern slopes are usually wetter than northern and eastern slopes because moisture laden air masses from the Pacific coast move into the high altitude mountains during the winter (Mosiño-Alemán and García 1974; Calvert et al. 1989). I observed that monarchs seem to be sensitive to changes in the relative humidity of the forest. When the relative humidity dropped below 35% for several days (Fig. 2.2), the monarch aggregation abandoned its location and re-formed in a more humid area with an average of 60% relative humidity (Alonso-M. unpublished data).

Previous studies also documented a massive movement of a monarch aggregation to a new location. As the overwintering period progressed, the entire aggregation departed from the original site and reformed on trees at lower altitudes (Calvert and Brower 1986; Calvert et al. 1989; Calvert 1994). By monitoring temperature, humidity, and rates of water evaporation throughout the overwintering period, I found three distinct climatic characteristics that were correlated with the movement of the monarch aggregation. I noted that the Llano del Toro overwintering aggregation departed from the study site when (1) the ambient temperature in the shade was above 16°C (Fig. 2.1), which is the upper limit of the monarch's thermal flight threshold (Masters et al. 1988; Alonso-M. et al. 1993); (2) the minimum relative humidity was below 35% for several days (Fig. 2.2); and (3) the rate of water evaporation was higher than 30 ml/week (Fig. 2.5). I

also observed that monarchs abandoned opened areas first, followed by monarchs clustered in closed areas. Monarchs usually reform their aggregations in sites with high relative humidity, such as above a stream with running water (Calvert and Brower 1986; A. Alonso-M. unpublished data).

Despite the mounting evidence indicating that logging is detrimental to the survival of overwintering monarchs (Calvert and Brower 1981; Calvert et al. 1982, 1986, 1989; Calvert and Cohen 1983; Brower and Malcolm 1991; Alonso-M. et al. 1992; Snook 1993; Anderson and Brower 1996), some argue that logging should be permitted in the core areas of the reserve (Hoth 1993; Chapela and Barkin 1995). This pressures government leaders to consider changing the presidential decree. In this chapter, I analyzed several microclimatic characteristics recorded in closed and opened areas of the Oyamel fir forest. I conclude that monarch butterflies need cool, but not freezing temperatures to preserve their lipid reserves. They also require relatively high humidities, but at the same time need to avoid the accumulation of water on their body surfaces which would cause death at temperatures below freezing. I recommend that the core areas of the MBSBR should be managed to provide a closed canopy with a natural understory vegetation. Further logging should not be permitted in the core areas of the MBSBR.

Table 2.1. Analysis of covariance (ANCOVA) comparing slopes and y-intercepts of several microclimatic variables registered in opened and closed areas during the 1993-1994 overwintering season at the Llano del Toro monarch butterfly aggregation, located at Sierra Chincua, Michoacan, México. The rate of change was not different in any of the comparisons (i.e. slope $p > 0.05$). In two comparisons I found a significant difference in the y-intercept. Warmer temperatures were registered in closed areas and higher wind speeds were registered in opened areas.

	F-value	d. f.	p-value
<u>Ambient Temperature</u>			
Minimum			
slope	0.30	1, 195	$p = 0.58$
y-intercept	58.0	1, 196	$p < 0.001$
Maximum			
slope	1.55	1, 194	$p = 0.22$
y-intercept	3.82	1, 195	$p = 0.06$
<u>Relative Humidity</u>			
Minimum			
slope	0.02	1, 194	$p = 0.89$
y-intercept	0.03	1, 195	$p = 0.88$
Maximum			
slope	0.29	1, 194	$p = 0.59$
y-intercept	0.70	1, 195	$p = 0.40$
<u>Wind Velocity</u>			
Maximum			
slope	3.44	1, 124	$p = 0.07$
y-intercept	66.1	1, 125	$p < 0.001$

Figure 2.1. Comparisons of average daily minimum and maximum ambient temperatures recorded in closed and opened areas at the Llano del Toro monarch butterfly aggregation during the 1993-1994 overwintering season. Day 40 refers to December 10 and day 140 refers to March 21, 1994. Differences between closed and opened daily minimum and maximum temperatures were calculated by subtracting temperatures recorded in opened areas from those recorded in closed areas. Data in C indicate that minimum temperatures were consistently colder in opened areas (average difference between closed and opened areas = 1.18°C). Data in D show that opened areas were increasingly hotter later in the winter (average difference between closed and opened areas after day 100 of overwintering = -0.60°C).

Figure 2.1

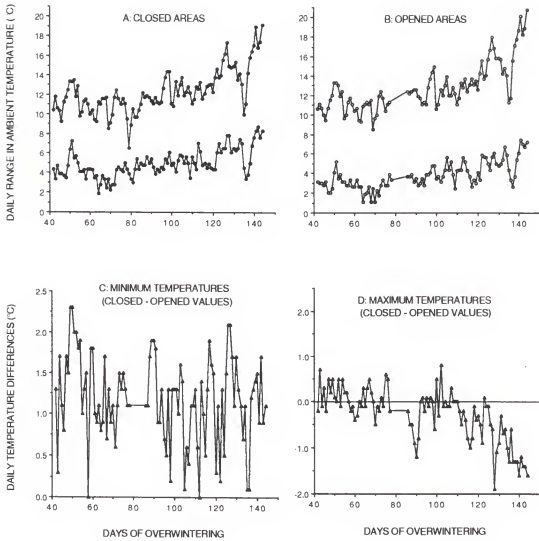


Figure 2.2. Comparisons of average daily minimum and maximum percent relative humidity recorded in closed and opened areas at the Llano del Toro monarch butterfly aggregation during the 1993-1994 overwintering season. Day 40 refers to December 10 and day 140 refers to March 21, 1994. Note that the relative humidity remained high during most of the overwintering season. When it dropped below 35% for a few days (dashed line in A and B), the monarch aggregation abandoned the location and reformed in an area near running water. Data in C and D indicate that minimum and maximum relative humidities were consistently higher in opened than in closed areas (average difference between minimum and maximum relative humidities in closed and opened areas = -2.1, and -2.2% respectively).

Figure 2.2

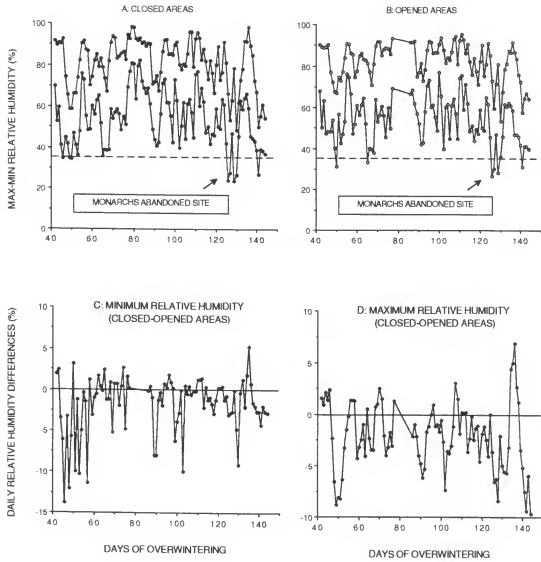


Figure 2.3. Comparisons of average daily maximum wind speed recorded in closed and opened areas at the Llano del Toro monarch butterfly aggregation during the 1993-1994 overwintering season. Day 40 refers to December 10 and day 140 refers to March 21, 1994. Differences between closed and opened daily maximum wind speed were calculated by subtracting wind speed recorded in closed areas from those recorded in opened areas. Values below zero indicate that lower wind speed values were recorded in closed quadrats.

Figure 2.3

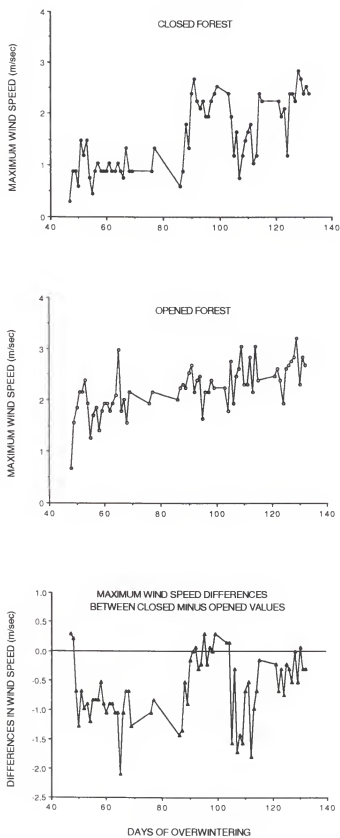


Figure 2.4. Diurnal changes in the average wind velocity during 64, 24 hr cycles in closed (dark circles) and opened (opened circles) areas. Data were gathered during the 1993-1994 overwintering season at the Llano del Toro monarch butterfly aggregation, located at Sierra Chincua, Michoacan, México. Each point is the average of 2-hourly values, with standard errors.

Figure 2.4

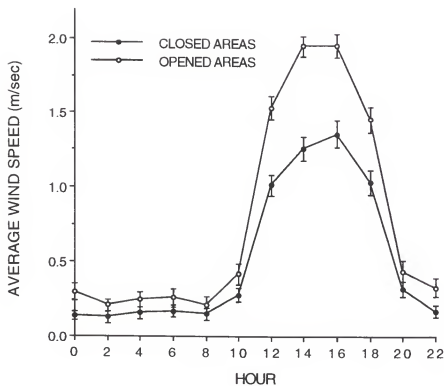


Figure 2.5. Comparison of the amount of water evaporated (ml) in closed and opened areas during the 1993-1994 overwintering season. Each bar (dark = closed; clear = opened) represents the average of the water evaporated at 10 sites/week (+ standard error). Day 57 refers to the week of December 20 to 27, 1993, and day 144 refers to the week of March 18 to 24, 1994. Water evaporated at a higher rate in opened (24.7 ml/week) than in closed (21.9 ml/week) areas. At 123 and 130 days, the monarch aggregation abandoned the study location and reformed down slope in a more humid area.

Figure 2.5

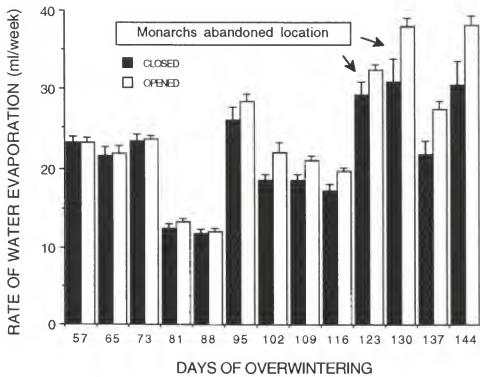
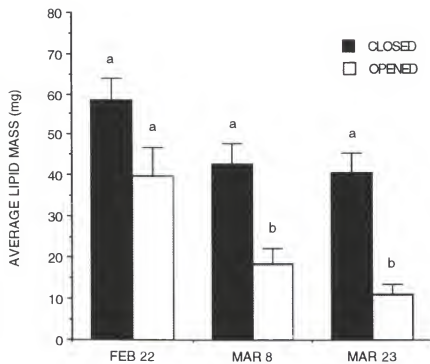


Figure 2.6. Monarchs held in experimental enclosures consumed their lipid reserves at a faster rate when they were exposed to the microclimatic conditions in opened areas. At the beginning of the experiment, on February 7, 1986, monarchs had an average lipid mass of 68 mg ($\pm$ 5.3). Monarchs in open areas metabolized lipids faster than monarchs in closed areas. I used the overall mean square error of the two way (treatment X date) ANOVA to compare means per date. Samples with different letters are significantly different (Ryan Q-test). Each bar represents the average of 20-30 butterflies with its standard error.

Figure 2.6



CHAPTER 3

USE OF LIPID RESERVES BY MONARCH BUTTERFLIES OVERWINTERING IN MEXICO: IMPLICATIONS FOR CONSERVATION

Introduction

Each year during the autumn in North America, tens of millions of monarch butterflies (Danaus plexippus L.) migrate to the mountains of central México. As their larval food source of milkweed plants (Asclepias spp.) diminishes in North America at the end of the summer, monarchs escape the cold northern winter and migrate to the cool, moist environment of high altitude mountain peaks. They arrive in early November and form dense aggregations in several areas in the Transverse Neovolcanic Belt in the states of Michoacán and México (Urquhart and Urquhart 1978a, b; Brower 1985; Calvert and Brower 1986; Calvert et al. 1989; Calvert and Lawton 1993). There, they remain largely inactive and maintain a state of reproductive diapause until March when they migrate back to the southern United States to exploit freshly emerging milkweeds (Herman 1985; Brower and Malcolm 1991; Malcolm et al. 1993).

Autumn migrant monarchs differ in behavior and physiology from monarchs during the rest of the annual cycle. Spring and summer generations form extensive breeding populations and individuals do not accumulate large lipid reserves (Beall 1948; Tuskes and Brower 1978; Brower 1985; James 1984; Walford 1980). In contrast, late summer and

autumn generations do not mature sexually and build up extensive lipid reserves as they migrate to overwintering areas in México (Beall 1948; Brown and Chippendale 1974; Walford 1980; Brower 1985; Masters et al. 1988; Calvert and Lawton 1993). These differences have also been found in monarch populations in California (Tuskes and Brower 1978; Chaplin and Wells 1982; Wells et al. 1993), and eastern Australia (James 1984, 1986, 1993). As they migrate southward, monarchs stop frequently to obtain nectar from wild flowers, which is then converted to lipids and stored for use during the overwintering period (Cenedella 1971; Brown and Chippendale 1974; Turunen and Chippendale 1980). Lipids are an efficient energy source for flying insects due to their relatively low weight and high energy content (Kozhantshikov 1938; Beenakkers et al. 1981). The accumulation of lipids before overwintering has been reported in other insects, including flies (Valder et al. 1969; Adedokun and Denlinger 1985), beetles (Hodek and Cerkasov 1961; Lambremont et al. 1964; Dortland and Esch 1979), other butterflies (Pullin 1987), and moths (Chippendale 1973; Gunn and Gatehouse 1986).

Lipid mass in monarchs declines slowly during the overwintering period due to the inactivity of clustered monarchs at low ambient temperatures (Chaplin and Wells 1982; Masters et al. 1988; Calvert et al. 1989; Leong 1990, Leong et al. 1991; James 1993). A small percentage of overwintering monarchs show active behaviors such as flying and gliding within the colony, as well as flights to nearby water and nectar sources (Masters et al. 1988). Downslope colony movement to more humid areas also occurs as the dry season progresses (Calvert and Brower 1986; Calvert 1994; Chapter 2). Since these behaviors consume the monarchs' limited lipid reserves, Masters et al. (1988) hypothesized that monarch butterflies

may try to avoid them in order to conserve energy. Lipid reserves remaining at the end of the overwintering period are used for migration and reproduction, and are probably supplemented by nectar feeding along the migration route (Heitzman 1962; Brower 1985; Urquhart 1987).

In México, monarchs overwinter in forests dominated by the Oyamel fir (*Abies religiosa* H.B.K.) where microclimatic conditions are suitable for their five month overwintering period (Calvert et al. 1989). These forests have island-like distributions on mountain peaks at elevations between 2,400 and 3,600 m (Rzedowski 1983). The restricted distribution of the Oyamel forest and increasing logging and clearing for agricultural fields make it more vulnerable to deforestation than any other forest type in México (Calvert et al. 1989; Snook 1993). Degradation of the forest endangers the migration phenomenon of the monarch butterfly (Brower and Malcolm 1991; Malcolm 1993; Anderson and Brower 1996). In 1986, the Monarch Butterfly Special Biosphere Reserve (MBSBR) was created by a presidential decree for protection of the monarch butterfly. It includes 16,110 ha of which 11,600 ha are classified as buffer zones where forest extractions are permitted (Diario Oficial 1986). Therefore, logging-free areas consist of only 4,500 ha. Notwithstanding the small size and protection status, the federal government is under pressure from land owners to change the presidential decree to approve logging permits in the core areas.

Hoth (1993) has recently advanced an argument to justify logging in the core areas of the monarch reserves. He hypothesized that tree extraction would create open areas where understory plants may produce more flowers than in closed areas. In theory, such increased availability of nectar resources upon which monarchs may feed could translate into fewer

monarchs depleting their lipid reserves and, therefore, more monarchs surviving the winter in México and successfully migrating the following spring. Brower and Malcolm (1991), however, found that monarchs visiting flowers during the overwintering period in January 1981 had lower lipid mass than did inactive monarchs clustered on trees. Their results suggest that flower-visiting monarchs may differ from clustered monarchs in their ability to overwinter and migrate successfully. Moreover, Brower (1995b) pointed out that the amount of nectar available within the area utilized by the butterflies during the winter would be inadequate even if the entire core areas were thinned.

In this chapter, I extended Brower and Malcolm's 1991 study to compare several other physical characteristics between flower-visiting monarchs and inactive monarchs clustered on trees. I compared 1) lipid mass, an indicator of monarch energy reserves; 2) water mass, which is important for survival and may drive monarchs to become active for rehydration; 3) lean mass, which is a reflection of body size and protein content; and 4) wing length, which is another indicator of body size. I hypothesized that the cohort of overwintering monarchs that visit flowers do so because they are attempting to replenish low lipid and/or water reserves. I also hypothesized that monarchs clustered on the trees beneath the fir forest canopy would have high lipid mass at the beginning of the overwintering period and then use those lipid reserves through the period at a rate not different from that expected based on their basal metabolic rate. This would indicate that monarchs do not move very much during the overwintering period and that nectar feeding is probably not important for clustered butterflies.

I compared the lipid reserves of the flower-visiting monarchs and the inactive monarchs clustered on trees to 1) autumn migratory monarchs collected in Texas on their way to the overwintering sites in México, 2) migrating monarchs that had successfully returned from the overwintering sites in México to the southern United States, and 3) reproductively active spring and summer generations collected in Wisconsin and Minnesota. I hypothesized that these monarch groups would differ in the quantity of their lipid reserves due to contrasting activity levels and reproductive states.

Methods

Winter Study Site

In México, monarchs form 9 - 12 overwintering aggregations near the summits of mountains in an area from the western slopes of Volcano Nevado de Toluca in the state of México, northwest to the city of Zitácuaro and north to the Altamirano mountain in the state of Michoacán (Figure 2 in Calvert and Brower 1986; Calvert et al. 1989; Calvert and Lawton 1993). The overwintering period overlaps with the dry season which extends from November to April. The area receives more than 1,000 mm of rain during the summer wet season (Rzedowski 1983).

This study was conducted on the southwestern facing slope of Zapatero Canyon at the Sierra Chincua, a mountain range in northeastern Michoacán (19°40'48"N and 100°17'54"W, Anonymous 1976). In the winter of 1993-1994, I studied the Llano del Toro overwintering monarch aggregation, a colony that has formed almost every year since the

overwintering sites were discovered in 1975 (Urquhart and Urquhart 1976). The forest is dominated by A. religiosa trees; other tree genera found in the area include Pinus L., Quercus L., and Buddleia L. (Soto and Vazquez 1993). The understory vegetation consists primarily of herbaceous and bushy plant species in the Asteraceae and Lamiaceae, with a diverse assortment of ascomycetes, basidiomycetes and bryophytes (Espejo et al. 1992).

Collections of Inactive Monarchs Clustered on Trees

Early in the morning, before ambient temperature was high enough to permit flight (9.9-16.1°C, Masters et al. 1988; Alonso-M. et al. 1993), I collected monarchs that were inactive, hanging immobile on the oyamel fir branches in overwintering clusters. Since no monarchs departed from the clusters before I made my collections, my samples are butterflies that spent the night clustered on the tree branches. With a pole attached to a standard butterfly net, I netted all butterflies within a cluster about four meters above the ground. I haphazardly selected 50 females and 50 males from the sample. Collections were made monthly on November 8 and December 5, 1993, and on January 10, February 15, and March 13, 1994. The mean number of butterflies per cluster was 265 (S.E. = 33.5, n = 5, range = 171 - 349). Butterflies were placed in individual glassine envelopes. They were immediately transported to a laboratory where I recorded right fore wing lengths, measured to the nearest 0.5 mm along the costal margin from base to apex, and wet mass to the nearest milligram, using a Sartorius 1205 MP electronic balance. Monarchs were then stored in a freezer at -4°C until chemical analysis was performed.

I dried the butterflies at 60°C for 16 hr, weighed them, and extracted lipids using the following method of Walford (1980) and May (1992). Each monarch was ground in a centrifuge tube in 20 ml petroleum ether with a Janke & Kunkel SDT Ultra Turrax tissuemizer. Each sample was then vortexed and placed in a medium speed shaker bath at 35-38°C for 30 min, with vortexing every 10 min. Tubes were centrifuged in a Dynac Clay Adams centrifuge at 1000 RPM for seven min, and the supernatant was decanted into preweighed aluminum pans on a 30°C hot plate. I added 15 ml of ether to the remaining solids in the centrifuge tube, and repeated the procedure described above. The weighing pans with the supernatant were allowed to evaporate for four hours to constant mass. The lipids were then weighed. I estimated water content as wet mass minus dry mass, and lean mass as dry mass minus lipid mass for each butterfly.

Collections of Flower-visiting Monarchs

I collected 50 females and 50 males visiting flowers on each of the following dates: December 12, 1993, January 13, February 12, and March 13, 1994. All butterflies were collected within a 300 m radius of the center of the 2.01 ha Llano del Toro overwintering colony. A monarch was considered to be visiting a flower only when it was perched on a flower or flower head and its proboscis was inserted into a flower. Lipid mass, lean mass, water mass, and wing length were recorded for each butterfly. I used the same methods as described above for the clustered butterflies.

Collections of Migrating Monarchs

For comparisons between overwintering and other monarch groups, I measured and analyzed the same characteristics in monarch butterflies

that were migrating through Texas to the overwintering sites in México. In October, 1993, 61 migrating females and 73 males were collected from transient clusters at Eagle Pass, Crystal City, and Castroville in south Central Texas.

I also compared these characteristics to monarchs that successfully migrated from México to the southern United States. Migrating monarchs can be identified by their cardiac glycoside fingerprint patterns, which reflect the specific cardiac glycoside content of the milkweed plants they fed on as larvae. Malcolm et al. (1993) found that 92% of monarch butterflies overwintering in México had fed as larvae on Asclepias syriaca, a milkweed species with a distribution in the northern United States. Any monarchs collected in the spring in the southern United States with similar cardiac glycoside fingerprint patterns would almost certainly be migrants returning to the United States from the overwintering sites in México. In April and May 1985, Malcolm et al. (1993) analyzed 134 monarchs collected in Texas, Louisiana, and Florida and found 110 migrant butterflies with the A. syriaca fingerprint pattern (49 females, 61 males). In April and May 1986, Malcolm et al. (unpublished data) analyzed 225 butterflies collected in Texas, Louisiana and Oklahoma and found 162 migrant monarchs with the A. syriaca pattern (75 females, 87 males). From Malcolm's et al. data (1993, unpublished), I selected spring migrants with the A. syriaca pattern that were collected before April 21 (83 females and 80 males) to evaluate lipid, water, and lean mass depletion during migration from México to the southern United States. I did not include later collections since data from those monarchs may show reproductive and aging effects. By comparing monarchs collected in different years, I am assuming that nectar available during the spring migration was the

same. I did not find differences in the mean fore wing length between clustered monarchs collected in March in 1985 and 1986 (Van Hook 1993), and clustered monarchs collected in March 1994 ($p > 0.05$).

I also compared the lipid and lean masses of my collections to several other published and unpublished samples of monarch butterflies, including freshly eclosed, summer breeders, and autumn and spring migrant monarchs (see Appendix).

Estimates of Lipid Utilization by Clustered Monarchs

Chaplin and Wells (1982) estimated resting metabolic rates for monarch butterflies. They studied energy budgets based on oxygen consumption at different ambient temperatures, ranging from five to 22°C, and determined that the resting metabolic rate for overwintering monarchs was related to thoracic temperature by the function

$$\log_{10} E = 0.048T_{th} - 0.368$$

where E = energy expenditure in joules per hour, and T_{th} = thoracic temperature in degrees Celsius. Following Masters et al. (1988), I used this function to estimate the expected energy consumed by inactive monarchs clustered on trees during the overwintering period. Since thoracic temperatures of inactive monarch butterflies beneath the forest canopy are not different from ambient temperatures (Chaplin and Wells 1982; Alonso et al. 1993), I used ambient temperatures that I recorded at the site as T_{th} .

I used on-site weather loggers (OWL, Electronically Monitored Ecosystems, 2229 Fifth St., Berkeley, CA 94710) to record ambient temperatures from December 12, 1993 to March 24, 1994. OWLs were

programmed to register the ambient temperature every minute and to record the mean of those measurements over two hr periods. I recorded the ambient temperature at six different closed canopy locations within the monarch colony. Temperature probes were placed near clustered butterflies at three meters above the ground. Data from the six locations were used to obtain mean ambient temperatures for the monarch colony every two hr. I then estimated the energy consumed by resting monarchs over two hour periods. I converted the energy spent to milligrams of lipid burned based on the energy yield from the oxidation of lipids as 37.66 joules/mg (i.e. 9.01 cal/mg; 4.18 joules/cal; Gordon 1977). From this, I calculated an expected amount of lipid that clustered monarchs burned each day.

I then compared my results to published rates of lipid consumption in two other overwintering populations of monarch butterflies. These included a population of western monarchs overwintering in California (Chaplin and Well 1982; Wells et al. 1993), and a population of monarchs overwintering in eastern Australia (James 1984).

Statistical Analysis

For each of my monarch collections, lipid mass, water content, lean mass, and wing length size data were first tested for normality using the Shapiro-Wilk W test (Zar 1984). I tested the means for homogeneous variances using Levene's test (Snedecor and Cochran 1980). Coefficients of determination (r^2 , Zar 1984) were computed to detect possible correlations between lipid mass, water content, lean mass, and wing length data.

Contrary to clustered monarchs ($p > 0.05$), lipid mass for flower-visiting monarchs was not normally distributed ($p < 0.001$; Fig. 3.1). The

variances were, however, homogeneous for both clustered and flower-visiting monarchs (Levene-median's test $F(7, 786) = 1.83, p > 0.05$).

Nonparametric Kruskal-Wallis one-way analyses of variance were used to test for differences among lipid mass and lipid index medians. The lipid index is computed as the lipid mass (mg) divided by the lean mass (mg) X 100 (Chaplin and Wells 1982; James 1984). It was determined to estimate the proportion of lipid mass to lean mass in each monarch and to facilitate comparisons to published studies. When comparisons yielded a significant Kruskal-Wallis statistic ($p < 0.05$), the lipid data were further analyzed by the Joint-Rank Ryan nonparametric test for stepwise unplanned multiple comparisons. For this test, I obtained the absolute value of the differences between the mean ranks of samples, and calculated the critical value by using the large-sample approximation (Day and Quinn 1989).

Parametric two-way analyses of variance (clustered and flower-visiting monarchs x month) were performed on water content, lean mass, and wing length data, after verifying that the means had homogeneous variances (Levene's mean test, $p > 0.05$, Snedecor and Cochran 1980). Comparisons yielding significant F values ($p < 0.05$) were further analyzed by the parametric Ryan's Q test for stepwise unplanned multiple comparisons. I used the Joint-Rank Ryan and the Ryan's Q test for multiple comparison tests because they are the best to control the experimentwise type I error rate (Day and Quinn 1989).

Based on the ambient temperatures that clustered monarchs experienced during the overwintering period, I calculated the amount of lipids consumed each day. I then compared this rate to the observed rate found for inactive and flower-visiting monarchs. I used analysis of covariance (ANCOVA, Zar 1984) to compare the slopes and the y-intercepts

of the rate of lipid loss between the rate of the expected and the observed lipid loss found for inactive butterflies clustered on trees. I also compared the observed and expected rate of lipid loss in flower-visiting monarchs.

All tests of significance were two tailed with a probability level for significance of 0.05, while all comparisons tested a null hypothesis of no difference. Statistics were computed using SuperAnova, Statview II, and JUMP statistical packages on a SE/30 Macintosh computer.

Results

Clustered and Flower-visiting Monarch Comparisons

Inactive monarchs clustered on trees had significantly higher amounts of lipid mass, water content, lean mass, and larger wings than the flower-visiting monarchs ($p < 0.001$; Figs. 3.1 and 3.2). The lipid index was also significantly higher for clustered monarchs, indicating that lipid mass differences between the two groups were not entirely due to monarch size (Kruskal-Wallis test, $p < 0.001$).

The physical condition of the clustered monarchs changed as the overwintering season progressed. At the beginning of the season, they had higher amounts of lipid mass, water content, and lean mass than at the end of the period (Figs. 3.1 and 3.2). Flower-visiting monarchs had low lipid mass values during the season, with water mass being lowest in the middle of the overwintering period ($p < 0.001$). No lean mass decline was found through time for flower-visiting monarchs ($p = 0.10$). Since I did not find wing length differences among individuals for either clustered ($p = 0.31$) or flower-visiting monarchs through time ($p = 0.09$), these results

suggest that the observed mass loss through time was not the result of size-related mortality or to the emigration of larger butterflies.

While the lipid mass of clustered monarchs was significantly correlated to water mass, lean mass, and wing size data, the coefficients of determination (r^2) accounted for less than 10% of the variability in those data (Table 3.1). For both clustered and flower-visiting monarchs, the highest coefficients of determination were found for wing size and lean mass data: the larger the butterfly, the higher the lean mass.

Comparisons to Migrating Monarchs

Monarchs migrating southward through Texas in October had lower amounts of water than did monarchs clustered on trees at the overwintering sites in November (Table 3.2). No differences in lipid mass ($p = 0.07$), lean mass ($p = 0.42$), or wing size ($p = 0.32$) were detected.

Comparisons of monarchs collected at the end of the overwintering period in March in México and monarchs collected in April in the southern United States, showed that clustered monarchs had higher lipid and lean masses, but not larger wings than migrant butterflies ($p < 0.001$; Table 3.2). Moreover, migrant monarchs had higher lipid mass and larger wings than flower-visiting monarchs ($p < 0.05$). No differences in water mass were found between the three groups ($p = 0.36$).

Non-migratory. Reproductive Active Generations

Lipid mass recorded from monarchs of the eastern and western populations in North America changed throughout the year (see Appendix; Fig. 3.3). Non-migratory, reproductively active generations of monarchs of the eastern population collected from April to August had mean lipid

amounts of about 20 mg, which is similar to mean values recorded for flower-visiting monarchs at the overwintering site in México (Fig. 3.1). Fall migrants in September and October increased their lipid amounts from about 60 and 80 mg respectively, to very high amounts (135 mg) when they arrived to the overwintering sites in November (Fig. 3.3). Both the eastern and western populations of clustered monarchs had high lipid masses at the beginning of the overwintering period that progressively decreased throughout the season.

Estimates of Lipid Utilization During the 1993-94 Overwintering Period

Using Chaplin and Wells' (1982) equation for basal metabolic rate and the ambient temperatures recorded at the overwintering colony during 1993-1994, I estimated that the resting metabolic rate of inactive monarchs within the winter clusters was on average 0.695 mg of lipid mass per day ($n = 103$ days, S.E. = 0.02). On December 5th, clustered monarchs had a mean lipid mass of 113 mg (S.E. = 4, $n = 100$). Since I started recording ambient temperatures on December 12th (i.e., day 42 of overwintering, November 1 = day 1), I used the calculated mean daily loss to estimate the expected lipid mass for that date based on the December 5th datum ($113 \text{ mg} - (0.695 \text{ mg} \times 7 \text{ days}) = 108.1 \text{ mg}$). I then used 108.1 mg of lipids as the initial datum from which I subtracted the expected amount calculated for each day (Fig. 3.4). For example, I expected monarchs to have 107.5 mg of lipids by day 43 of overwintering ($108.1 - 0.596$, estimated amount consumed on day 43). The expected rate of lipid loss of clustered monarchs followed the linear regression model

$$\text{lipid mass (mg)} = 138.2 - 0.669 (\text{days of overwintering}),$$

with an $r^2 = 0.99$ (ANOVA $F(1, 102) = 196.9$, $p < 0.001$). I then regressed the observed monthly mean lipid content of clustered monarchs, and obtained the following linear function

$$\text{lipid mass (mg)} = 138.4 - 0.653 (\text{days of overwintering}),$$

with an $r^2 = 0.95$ (ANOVA $F(1, 4) = 61.2$, $p < 0.01$). The slopes of the expected and observed functions are not significantly different from each other (ANCOVA $F(1, 104) = 0.6$, $p = 0.44$; Fig. 3.4). From these data, it is clear that inactive monarchs clustered on trees lost their lipids passively in relation to the ambient temperature, as would be expected based on their resting metabolic rate.

Rates of Lipid Utilization in México, California and Australia

I compared the rate of lipid utilization found for clustered monarchs in México to published rates of lipid use in two other overwintering locations, in California (Chaplin and Wells 1982; Wells et al. 1993) and Australia (James 1984). I found that the rates of lipid use were not significantly different among the three sites (ANCOVA $F(2, 8) = 0.08$, $p = 0.92$; Fig. 3.5). These data suggest that monarchs from these three distant locations remain largely inactive during the overwintering period and that overwintering temperatures are similar. I also found that monarchs in México have higher lipid mass (133 mg) at the beginning of the season, than butterflies in California (84 mg) and eastern Australia (94 mg), and that monarchs departed at the end of the season from the three sites with similar mean lipid masses: México 56, California 57, and Australia 53 mg

(see Appendix). Note that the mean lipid mass for flower-visiting monarchs in March was only 21 mg.

Proportion of Clustered Monarchs That May Need to Visit Flowers

I estimated the proportion of inactive monarchs clustered on trees that may need to visit flowers as they deplete their lipid reserves during the overwintering period. For each monthly collection ($n = 100$ butterflies), I determined the number of clustered monarchs that had lipid amounts lower than the average lipid mass plus one standard deviation recorded for flower-visiting monarchs. For example, in December, this value for flower-visiting monarchs would be 97 mg (average = $53 + 44$, S.D.). For monarchs clustered on trees, 41% had lipid masses lower than 97 mg.

I also determined the number of flower-visiting monarchs that had higher lipid amounts than the average lipid mass found for clustered monarchs, minus one standard deviation. In December, clustered monarchs had on average 113 mg of lipid mass (S.D. = 44). I thus determined that 34 flower-visiting monarchs (34%) had a higher lipid mass than 69 mg. I performed a similar analysis for January, February, and March. I combined data for the 4 months and obtained an average of 26%. In other words, of 400 flower-visiting monarchs, 104 had lipid amounts greater than the monthly average recorded for clustered monarchs minus one standard deviation. Of 394 clustered monarchs, 120 (30.5%) had lipid amounts lower than the monthly average plus one standard deviation recorded for flower-visiting monarchs. I thus propose that about 30% of the overall population of inactive monarchs clustered on trees from December to March may depart from roosting locations to visit flowers.

Discussion

Clustered and Flower-visiting Monarch Comparisons

Fir forests provide the microclimate needed for successful overwintering in México. In November, inactive monarchs clustered on trees had an average lipid mass of 133 mg. These monarchs consumed their lipid reserves in relation to the ambient temperature (Chaplin and Wells 1982), such that by the middle of March, just prior to departure from the overwintering site, they had an average of 56 mg of lipids, a 57.9% lipid loss in 5 months (Fig. 3.4). Monarchs that successfully migrated to the southern United States in April had on average 26 mg of lipid mass, a further loss of 22.6%, indicating that migration to the United States is energetically expensive.

In contrast, monarchs that visited flowers at the overwintering sites had highly depleted lipid reserves. In December, they had an average lipid mass of 53 mg, 47% of that of clustered butterflies, and the same average as clustered monarchs in March (56 mg). Moreover, during the last three months of the overwintering period, the frequency distributions of the lipid mass data for flower-visiting monarchs showed a high proportion of butterflies with lipid masses close to zero (Fig. 3.1). Since monarchs convert carbohydrates from nectar into lipids within a few hours after ingestion (Cenedella 1971), and I found very few butterflies with medium or high lipid levels visiting flowers, I suggest that either (1) flower-visiting monarchs maintained their lipid reserves at low levels by visiting flowers but were unable to reach levels found in clustered monarchs, or (2) that flower-visiting monarchs starved to death, and were replaced by clustered

monarchs that departed from their roosting trees to visit flowers when their lipid reserves dwindled to critical levels. The frequency distribution of clustered monarchs shifted toward lower lipid mass categories as they consumed their lipid reserves during the season (Fig. 3.1). Clustered monarchs with low lipid reserves of about 20 mg (± 20) may have departed from their roosting trees to visit flowers. Monarchs that recently departed from clusters could still have high levels of water mass, as I found for flower-visiting monarchs in February and March (Fig. 3.2). I then suggest that as the season progressed and clustered monarchs consumed their lipid reserves, monarchs with low lipid masses departed from clusters to visit flowers. I also acknowledge that some flower-visiting monarchs could cluster. However, only 26% had lipid mass (minus one standard deviation) of that recorded for clustered monarchs. My data suggest that about 30% of the overwintering population may become flower-visiting monarchs.

I found consistent physical differences between clustered and flower-visiting monarchs (Fig. 3.2). Clustered butterflies had higher lipid mass, higher water content, higher lean mass, and larger wings than flower-visiting monarchs throughout the season. Since few flower-visiting monarchs had characteristics similar to clustered monarchs, they either (1) stayed near the overwintering aggregation but did not cluster in trees, (2) returned to the overwintering aggregation but clustered in higher positions within the fir trees where I could not collect them, (3) departed early from the monarch aggregation, or (4) died at the overwintering sites.

Several lines of evidence support my hypothesis that flower-visiting monarchs in March are in such poor condition that they may not be able to migrate to breeding areas in the southern United States. First, migrating monarchs collected in April in Texas and Louisiana had significantly

higher amounts of lipid mass than flower-visiting monarchs collected in March at the overwintering site, an indication that flower-visiting monarchs may not have enough lipid reserves to migrate back to the United States successfully (Table 3.2). Second, migrating monarchs arrived in the southern United States with less than 50% of the lipid mass and less than 10% of the lean mass found in clustered butterflies in March, but they had the same wing length size, suggesting that migration is energetically expensive, and that this collection of butterflies came from clustered monarchs in México (Table 3.2). Third, flower-visiting monarchs may be in a different physiological state than clustered monarchs. They had lipid reserves (20 mg) similar to those found in non-migratory summer breeders (Fig. 3.3). Moreover, Van Hook (1993) described physical characteristics of male monarchs that became reproductively active during the overwintering period. She found that they had low wet mass, and small wings that were in poor condition (i.e. less scales, broken wings), which are characteristics that closely resemble those of flower-visiting monarchs. Reproductively active monarchs have high levels of juvenile hormone, a hormone that controls development of reproductive organs, and utilization of lipids, and accelerates aging in monarch butterflies (Barker and Herman 1976; Dallman and Herman 1978; Lessman and Herman 1983; Herman 1985; Herman et al. 1989).

It therefore appears that flower-visiting monarchs do not have enough lipid reserves to migrate back to the breeding areas of the southern United States. My analysis supports the hypothesis that this cohort of butterflies is derived from the clustering butterflies that arrived in México with low lipid levels. By opening the forest, ambient temperatures will increase and hence so will the metabolic rate of clustered monarchs

(Chapter 2). Thus, a higher percentage of the population would enter the cohort of butterflies with low lipid contents. My data argue that there is no need to create open areas in the core zones of the MBSBR to promote the production of flowers for overwintering monarch butterflies. All indications are that this would make matters worse, not better.

Detrimental Effects of Logging on Monarch Survival

Data presented in this chapter strongly support the hypothesis that the overwintering success of monarch butterflies is not a matter of replenishing their lipid reserves by visiting flowers. Instead, success is dependent upon how well monarchs conserve their lipid reserves under the appropriate microclimatic conditions. There is abundant evidence for detrimental effects that logging has on overwintering monarch survival in relation to microclimate. Calvert and Brower (1981) and Calvert et al. (1982) showed that clustered monarchs freeze to death more often in thinned forest than monarchs in closed canopy forest. In 1981, 2.5 million butterflies died during a period of extreme cold weather (Calvert et al. 1983). Similarly, in 1992, about 83% of the Herrada monarch colony perished (Culotta 1992). Overwintering monarchs can survive temperatures several degrees below freezing because their body fluids can supercool (Lee 1989; Anderson and Brower 1993, 1996; Larsen and Lee 1994). However, colder temperatures registered in thinned forests promote the accumulation of dew on the body surface of monarchs and wetting during storms (Calvert and Brower 1981; Anderson and Brower 1996). When the external moisture freezes, ice crystals penetrate the cuticle and promote ice nucleation in the haemolymph, killing the butterflies (Alonso-M. et al. 1992; Anderson and Brower 1993; Larsen and Lee 1994). Thinned forests also increase the

intensity of bird predation on monarchs (Brower and Calvert 1985; Chapter 5), and greater exposure to sunlight increases butterfly activity and lipid use (Chaplin and Wells 1982; Masters et al. 1988). In fact, monarch colonies such as those at Altamirano, San Andres and Chivati-Huacal (map in Calvert and Brower 1986) have most likely changed in response to low tree densities resulting from current and past forest extractions. Over time, these colonies have become smaller, ephemeral, unstable and in several years absent (W. H. Calvert unpublished data). Similar observations have been recorded from degraded forest groves where monarchs overwinter in California (Weiss et al. 1991).

Management Recommendations and Conclusions

Core areas of the monarch reserve are already subject to numerous human activities. Out of the 4,500 logging-free hectares of the reserve, 1,000 are being used as centers for ecotourism, one of the major alternative incomes to land owners. Fifteen hundred hectares have been illegally logged and the remaining 2,000 ha have a mosaic of closed and open forest patches where most of the scientific research on the migrating monarchs and on the oyamel forest is conducted (A. Alonso-M. unpublished data). Since the core areas the MBSBR are small, for them to serve as sources of plant and animal species for recolonization of the 11,600 ha of the buffer zone, they should be increased in size and maintained as logging-free areas. Ideally, unprotected overwintering aggregations should be incorporated into the MBSBR so that the risk of extinction of the endangered phenomenon of the monarch butterfly migration will decrease.

The understory vegetation of the oyamel fir forest also plays an important role in the survival of overwintering monarch butterflies. Many

clustered monarchs are dislodged from tree trunks and tree branches during storms and by predatory birds at ambient temperatures when monarchs are unable to fly (Brower and Calvert 1985; Masters et al. 1988; Arellano et al. 1993). Monarchs on the ground shiver and crawl up onto nearby vegetation (Alonso-M. et al. 1993) to avoid freezing ground temperatures (Calvert and Cohen 1983; Alonso-M et al. 1992) and mouse predation (Glendinning et al. 1988). To date, conservation and management strategies have not emphasized protecting the understory vegetation. It is currently being damaged by logging practices, cattle grazing, and trampling by tourists (Calvert et al. 1989; Snook 1993). Future management must also minimize impact on the oyamel tree seedlings and on the understory vegetation.

Results from this study demonstrate that it is ill-advised to thin fir forests to create nectar sources. Based on the rate of lipid loss throughout the overwintering period, monarch butterflies need intact closed forest for successful overwintering. The overall low rate of lipid mass loss by inactive monarchs clustered on trees does not support the argument favoring artificial openings by logging in the monarch reserves. Management strategies for the conservation of the endangered migration phenomenon of the monarch butterfly should totally preclude logging practices in core areas of the reserve.

Table 3.1. Coefficients of determination (r^2) for correlations between lipid mass, water content, lean mass and wing length data for monarch butterflies overwintering in México. Correlations for inactive monarchs clustered on trees are shown in the right part of the matrix, while flower-visiting monarchs are in the left.

	<u>LIPID</u>	<u>WATER</u>	<u>LEAN</u>	<u>WING LENGTH</u>
<u>LIPID</u>	---	0.10*	0.08*	0.04*
<u>WATER</u>	0.01 ^{ns}	---	0.33*	0.31*
<u>LEAN</u>	0.01 ^{ns}	0.30*	---	0.50*
<u>WING LENGTH</u>	0.02 ^{ns}	0.19*	0.65*	---

* significant positive correlations $p < 0.001$

Table 3.2. Comparisons between migrant monarchs through Texas in October (1993) and early arrivals at the overwintering sites in November (1993) in México. I also compared migrant monarchs collected in the southern United States in Texas-Louisiana-Oklahoma-Florida before April 21 (1985, 1986) to monarchs at the overwintering sites in México about to migrate back to the United States in March, 1994. Mean lipid mass, water content, and lean mass (mg), and right fore wing length (mm) data are shown, with standard errors in parentheses. Samples with different letters in columns are significantly different (Joint-Rank Ryan test for lipid samples and Ryan Q-test for other samples).

	<u>N</u>	<u>LIPID</u> ¹	<u>WATER</u> ²	<u>LEAN</u> ²	<u>WING LENGTH</u> ²
MIGRANTS VS OVERWINTERING					
Texas Oct	132	120 (6) ^a	281 (4) ^a	172 (3) ^a	52.4 (0.2) ^a
México Nov	100	133 (5) ^a	303 (4) ^b	170 (3) ^a	52.3 (0.2) ^a
OVERWINTERING VS MIGRANTS					
Inactive Mar	94	56 (3) ^a	261 (4) ^a	168 (2) ^a	52.1 (0.2) ^a
Flowers Mar	100	21 (2) ^b	246 (4) ^a	157 (2) ^b	51.3 (0.2) ^b
Southern USA	163	26 (2) ^c	252 (6) ^a	148 (2) ^c	51.9 (0.2) ^a

¹= Kruskal-Wallis test; ² = ANOVA.

Figure 3.1. Lipid mass frequency distributions of clustered and flower-visiting monarchs during 4 months of the 1993-1994 overwintering season in the Sierra Chincua, Michoacán, México. Mean values (mg), standard deviations (S.D.), and sample sizes (N) are given for each distribution. The intervals of the histograms are 0-9, 10-19, etc.

Figure 3.1

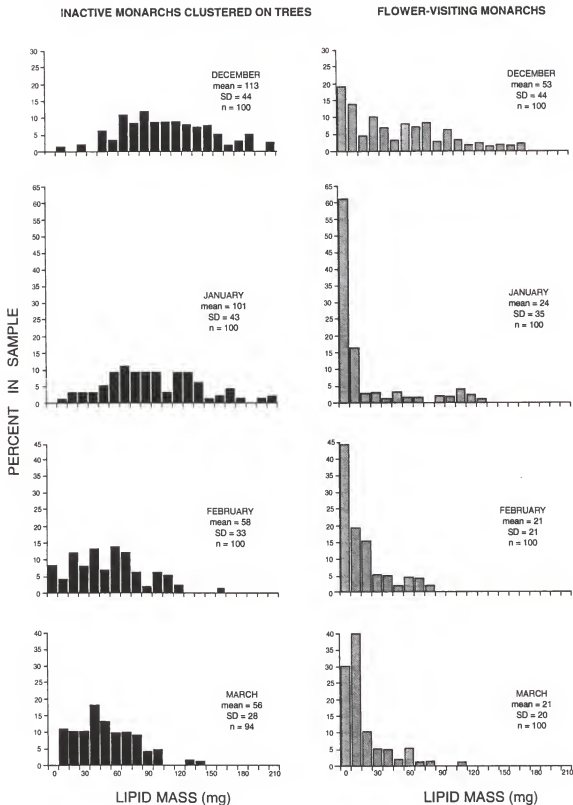


Figure 3.2. Mean lipid, water, and lean masses (mg), and wing size length (mm) of clustered (dark bars) and flower-visiting monarch butterflies (light bars) during 4 months of the 1993-1994 overwintering season. Samples with different letters are significantly different (Joint-Rank Ryan test for lipid samples and Ryan Q-test for other samples). Each bar represents the average of 100 butterflies with its standard error.

Figure 3.2

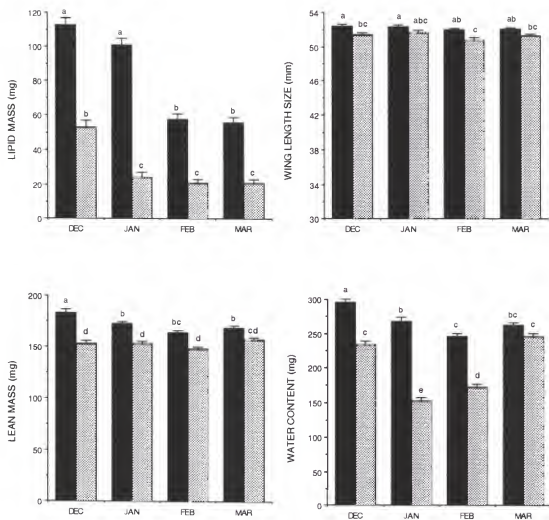


Figure 3.3. Lipid mass changes of 5211 monarch butterflies collected by many researchers over several years at different times during their annual life cycle. Data for the eastern North American population, and flower-visiting monarchs are presented. Numbers indicate the total number of sample collections used to obtain the average ($\pm$ S.E.) for each month. Refer to the Appendix to consult original source of data. Note that non-migratory spring and summer generations have a mean value of 20 mg of lipid mass, the same value recorded for flower-visiting monarchs.

Figure 3.3

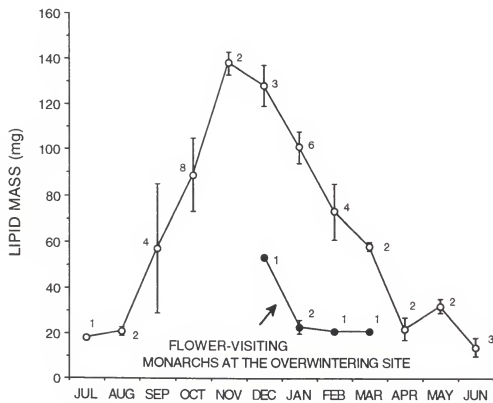


Figure 3.4. Comparison between expected and observed lipid mass loss for monarchs overwintering in México. Points with standard errors (straight bars) are the average of 100 butterflies (50 females and 50 males). Day 1 refers to November 1, 1993 and day 150 to March 31, 1994. The regression line of lipid mass for clustered monarchs (dark dots) against time is plotted. The expected lipid loss was estimated from the ambient temperatures that monarchs experienced during the season (see text). Notice that the 2 lines virtually overlap. At day 42, flower-visiting monarchs (clear dots) had less than half of the lipid resources of the clustered monarchs, and by day 74 through day 140, lipids stabilized at about 20 mg. Clustered butterflies lost their lipids as predicted but did not drop below 50 mg, 250% higher than the butterflies that visited flowers.

Figure 3.4

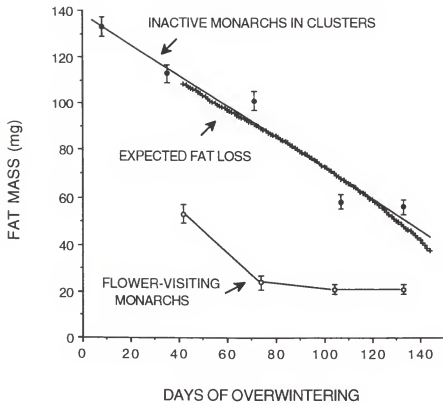
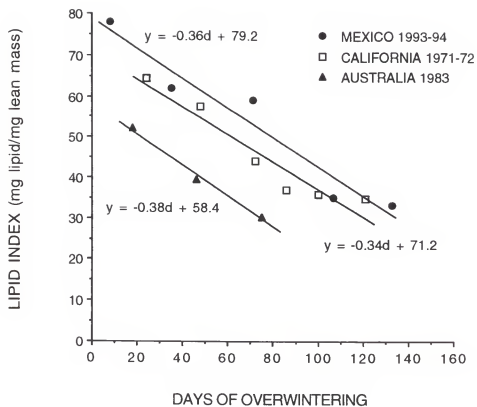


Figure 3.5. Lipid indices for overwintering monarch butterflies through time. Monarchs from Australia (James 1984), California (Chaplin and Wells 1982), and México (this chapter) have the same rate of lipid loss during the overwintering period.

Figure 3.5



CHAPTER 4

CONSERVATION IMPLICATIONS OF FLOWERING PLANT AVAILABILITY FOR OVERWINTERING MONARCH BUTTERFLIES IN MEXICO

Introduction

Each year the North American population of monarch butterflies (Danaus plexippus L.) east of the Rocky Mountains migrates to several mountain peaks in central México. These overwintering sites were first described by F. A. Urquhart and colleagues after several decades of research on monarch migration (Urquhart 1976; Urquhart and Urquhart 1976). Interested in the preservation of the spectacular monarch aggregations, the Urquharts decided not to share the exact location of their findings (Brower 1995a). Despite this, the publication of popular and scientific articles on monarchs overwintering in México soon made the sites known. Ten million monarchs per hectare were difficult to hide (Urquhart 1976; Brower 1977, 1985; Barthelemy 1978; Urquhart and Urquhart 1978a, b; Calvert et al. 1979; Calvert and Brower 1986).

W. H. Calvert and L. P. Brower, from the University of Florida, conducted pioneering research on the biology of monarch butterflies overwintering in México. They soon realized that the Oyamel fir trees (Abies religiosa H. B. K.), upon which monarchs roost during the overwintering period, were being commercially exploited at a fast rate. They determined that the survival of overwintering monarchs was closely

related to the microclimate registered in closed canopy forests, such that creating open areas by logging enhanced monarch mortality (Calvert and Brower 1981; Calvert and Cohen 1983; Calvert et al. 1982, 1983).

In 1986, the Monarch Butterfly Special Biosphere Reserve (MBSBR) was created by the president of México for protection of the monarch butterfly (Diario Oficial 1986). Five reserves located on mountain peaks in the Transverse Neovolcanic Belt in the States of México and Michoacán constitute the MBSBR (Calvert and Brower 1986; Calvert et al. 1989). The MBSBR includes 16,110 ha of which 11,600 ha are classified as buffer zones where forest extractions are permitted (Diario Oficial 1986). Therefore, wilderness logging-free areas consist only of 4,500 ha. Core areas were created for protection of the animal and plant species found in the relict Oyamel Fir forests, and to serve as sources of species to recolonize logged areas in the buffer zones. The restricted distribution of the Oyamel forest and increasing pressure from logging and clearing for agricultural fields make it more vulnerable to deforestation than any other forest type in México (Calvert et al. 1989; Snook 1993).

In this chapter, I present data to refute one of the arguments currently used to promote logging in the core areas of the MBSBR. Hoth (1993) has recently suggested that monarchs need to feed on nectar during the overwintering period, and that the core areas of the reserve do not have open areas where the butterflies can find plants in flower. He then hypothesized that logging would be beneficial for monarchs, since understory plants growing in logged-opened areas may produce more flowers than in closed areas. He assumed that with nectar readily available during the overwintering period, fewer monarchs would deplete

their lipid reserves. This hypothesis has been rapidly accepted by politicians and rural developers (Chapela and Barkin 1995).

To investigate whether increased logging is needed in the core area of one of the most pristine overwintering sites of the MBSBR (Calvert et al. 1989; Calvert and Lawton 1993), I looked at the distribution of flowering plants in existing closed and opened areas.

Methods

Study Site

I studied the Llano del Toro 1993-1994 overwintering monarch aggregation located in Sierra Chincua, a mountain range in northeastern Michoacán, México (19°40'48"N and 100°17'54"W, Anonymous 1976; see map in Calvert and Brower 1986). Monarchs select high altitude oyamel fir forests (3,000 m) where they find cool temperatures and high relative humidity needed for successful overwintering (Masters et al. 1988; Calvert et al. 1989). The understory vegetation consists primarily of plants in the Asteraceae and Lamiaceae plant families (Espejo et al. 1992; Soto and Vazquez 1993).

In addition to logging, local inhabitants obtain a limited number of non-timber products from the oyamel forests. These include the collection of flowers for religious rituals, herb plants for medicinal purposes (e.g. "te de monte" from Satureja macrostema, Lamiaceae), the extraction of resin from pine trees, and the harvesting of mushrooms during the rainy season. Non-commercial, domestic use of wood in the area includes fuelwood and charcoal production, beams for housing construction, and the production of

shingles. Free-ranging livestock are commonly found in the area and seem to have a negative effect on the survival of oyamel fir tree seedlings (Calvert et al. 1989; Snook 1993).

Plant Species in Flower and Monarch Butterfly Use

Since butterflies require a proboscis as long as the corolla tube to successfully obtain nectar from a flower (May 1992), I measured the proboscis length of 50 female and 50 male monarchs to the nearest 0.5 mm by extending their proboscis on a 15 cm ruler. I compared these monarch proboscis measurements to data on flower corolla length from Sanchez (1986). I also followed Sanchez' nomenclature to determine plant species.

Determination of Forest Openness

I first determined the degree of openness within the forest where monarchs formed their overwintering aggregation. From the center of the monarch aggregation, I ran four line transects (505 m each) directed 90° apart from one another. During most of the overwintering period, monarchs did not fly further than a 500 m radius from the studied 2.01 ha colony. Data were gathered at the end of March 1994. Each line transect was subdivided into 101, five meter stations that I defined as my sampling units ($n = 404$). Each station was subdivided into quarters from which I estimated the degree of canopy coverage. I classified the stations into six canopy openness categories: A) Naturally Closed: the station was 75-100% covered with canopy vegetation; B) Partially Closed: 50% of the station was covered with canopy and 50% was opened by natural causes. I considered natural causes of canopy openings to be dead standing or fallen trees, streams and creeks, rocky outcrops, and/or natural meadows dominated by

Potentilla candicans H. and B. Rosaceae (Soto and Vazquez 1993); C)

Partially Opened: 50% of the station was covered with canopy and 50% was opened by artificial causes. Opening by artificial causes was evidenced by human activities such as logging roads, tree stumps, and/or property boundary delimitations (see figures in Brower and Calvert 1985; Snook 1993); D) Naturally Open: more than 50% of the station was opened by natural causes; E) Open: 50% of the station was opened by natural causes and 50% was opened by artificial causes; F) Artificially Opened: more than 50% of the station was opened by artificial causes. At each station I recorded the most abundant flowering plant species that monarchs can use for nectaring.

Statistical Analysis

I determined the number of stations and the number of plant species in flower for each of the six canopy openness categories (Table 4.1). For the statistical analysis I combined categories A-C and called them "closed", while the combined data of categories D-F were called "opened". Using a G-test (Sokal and Rohlf 1995), I examined whether there was an association between the species of plants in flower and the habitat where there were found (i.e. closed versus opened sites). I also used a G-test to examine if each of the six most common flowering plant species (Table 4.1) were found more frequently either in closed or opened sites.

Results

Based on the matching of flower morphology and corolla length to the proboscis of overwintering monarchs, I found that there were six species of plants that had longer corolla lengths than the proboscis of monarchs. These include Salvia elegans Vahl. (corolla length = 20-35 mm), S. cardinalis H. B. K. (25-40 mm), Satureia macrostema (Benth.) Briq. (25 mm), Stachys coccinea Jacq. (20-25 mm) (Lamiaceae), Castilleja arvensis Benth. (30-35 mm, Scrophulariaceae), and Lupinus elegans (nectar is inaccessible due to flower morphology, Fabaceae; Sanchez 1986). These six species were found in 25% of the stations. The average proboscis length of overwintering monarchs was 15.9 mm (S.E. = 0.09, $n = 100$). Male monarchs had significantly longer probosces than females (male average = 16.2 mm, S.E. = 0.11, $n = 50$; female average = 15.6, S.E. = 0.11, $n = 50$, $p < 0.001$). Further analysis did not include these species.

I found that the core area of the Sierra Chincua reserve has many canopy openings (Table 4.1). Forty-three percent of the stations had some degree of human disturbance (categories C, E, and F), 41% of the stations were naturally closed (A), and 16% were open or partially opened by natural causes (B and D). These percentages are based on data from 399 stations. I originally had 404 stations but three stations did not have plants in flower; one station could not be placed in any of the categories since it was 1/4 naturally open, 1/4 artificially opened and 50% closed canopy.

I found a total of 21 plant species flowering in the area, including 14 in the Asteraceae and four in the Lamiaceae. Ninety-nine percent of the stations had plants in flower and most of the species were found in both closed (17 species) and opened (18 species) sites (Table 4.1). There was not a

significant difference between closed and opened sites in the number of plants observed flowering (G-test = 0.01, d. f. = 1, $p = 0.90$). I used the eight most common species (i.e. those that were found more than 10 times in the stations, Table 4.1) to test if they occurred more frequently in closed or opened sites. I found that Viola grahami Benth. (Violaceae) and Senecio prenantoides A. Rich (Asteraceae) occurred more frequently in closed sites (A-C), while Bidens triplinervia H. B. K. (Asteraceae) occurred more frequently in opened stations (D-F; G-test = 41.0, d. f. = 7, $p < 0.001$). No significant difference was found for the other five species between closed and opened sites.

Discussion

This study showed that the core area of the Sierra Chincua MBSBR already has a substantial number of areas with human-induced disturbances. Logging extractions that occurred before the MBSBR was created produced artificial openings in the forest such that 43% of the stations in this study had some degree of disturbance (Table 4.1). Thus, contrary to Hoth's (1993) observation that the core areas of the MBSBR do not have evidence of human disturbances, in this chapter I showed that the core area of the Sierra Chincua already has many artificial openings, even though it is considered one of the most pristine overwintering sites (Calvert et al. 1989; Calvert and Lawton 1993). Additional field observations indicate that it may take several decades for the Oyamel forest to recover from human perturbations. High altitude ecosystems tend to have low primary productivity compared to middle elevation pine-oak forests due to either low

ambient temperatures or restricted rainfall (Whittaker and Niering 1975; Stephenson 1990). Low ambient temperatures prevail during most of the year at the Oyamel fir forest, although it receives more than 1,000 mm of rain during the summer wet season (Rzedowski 1983). Moreover, the steep slopes of the mountains are susceptible to high rates of erosion during the rainy season when logging occurs in the buffer zones of the MBSBR (Madrigal 1967; Manzanilla 1974; Snook 1993).

My data showed that closed and opened areas had the same plant species composition (Table 4.1), and that there were plants in flower in most of the stations (99%). It has been shown that the distribution and abundance of plants of the understory vegetation in forest ecosystems is determined by complex above- and belowground processes (Anderson et al. 1969; Wilcox et al. 1981; Alaback 1982; McCune 1986), thus, further research should investigate the density of plants in flower in closed and opened areas, and their nectar production. In a recent paper, Riegel et al. (1992) reported that the increase of sunlight reaching the forest floor did not affect the production of understory plant biomass in a pine forest. They concluded that belowground resources (e.g. water and nitrogen) were the limiting factors. Further studies are needed to determine the factors involved in flower production and plant species distribution and abundance in the Oyamel forest ecosystem.

I now present a summary of a large body of data that provides additional arguments against opening the forest in the core areas of the MBSBR for the production of nectar for monarch butterflies. By thinning the forest, there is increased mortality due to freezing and bird predation. Furthermore, the consumption of lipids needed for their return migration to the southern United States would increase.

Calvert et al. (1982) reported colder nightly temperatures in areas that were thinned by logging and suggested that monarchs perched in opened areas could experience higher risks of freezing mortality especially during periods of extremely cold weather. There are published records of snow storms that killed 42% of the Sierra Chincua overwintering aggregation in 1981 (Calvert et al. 1983), and 6% in 1995 (E. Rendón-S., unpublished data). Eighty-three percent of the Piedra Herrada overwintering aggregation perished in 1992 (Culotta 1992). On these occasions, monarchs clustered in areas of closed forest remained dry and thus were better protected than those perched in opened areas (Anderson and Brower 1996). Wet monarchs die at warmer ambient temperatures (-4°C instead of -8°C) when ice crystals form on their body and trigger internal ice nucleation (Alonso-M. et al. 1992; Anderson and Brower 1993; Larsen and Lee 1994).

Thinned forests also increase the risk of bird predation on monarchs. Two independent studies have shown that monarchs were preyed upon more frequently in open than in closed areas (Brower and Calvert 1985; Chapter 5). Also, by opening the forest, a greater exposure to sunlight may increase butterfly activity and lipid use (Chaplin and Wells 1982; Masters et al. 1988). In chapter 2, I showed that monarchs that were experimentally maintained in opened areas consumed their lipid contents three times faster than those in closed areas. In fact, the San Andres overwintering monarch aggregation that forms outside the limits of the MBSBR protected area (map in Calvert and Brower 1986), and the highly disturbed Altamirano and Chivati-Huacal monarch aggregations have become smaller, ephemeral, unstable and in several years absent (W. H. Calvert unpublished data). These changes seem to be in response to low tree

densities resulting from current and past forest extractions. Figure 4.1 shows the range in ambient temperatures that monarch butterflies clustered in areas of closed forest experienced during the 1993-94 overwintering season at the Sierra Chincua. Temperatures were cool and stable during the period such that most monarchs remained quiescent, slowly consuming their lipid reserves. Low ambient temperatures also prevent monarchs from producing juvenile hormone, a hormone involved in sex organ maturation, lipid utilization, and aging (Barker and Herman 1976; Dallman and Herman 1978; Lessman and Herman 1983; Herman 1985; Herman et al. 1989).

Changing the presidential decree to approve logging in the relatively small and already impacted core zones of the MBSBR will be detrimental in at least three aspects. First, the objective of having core areas as a source of species for the buffer zones in a Biosphere Reserve will be lost (Diario Oficial 1986). Second, the economic input to the local economies will be ephemeral. Appropriate use of forest resources extracted from the buffer zones is lacking. Excessive middleman operations, high waste of wood, lack of forest plantations, and illegal logging do not allow economic development for land owners that request the permits (Calvert et al. 1989; Snook 1993). Third, altering the microclimatic conditions of the forest has resulted in higher mortality for overwintering monarchs (Calvert and Brower 1981; Calvert and Cohen 1983; Calvert et al. 1982, 1983; Alonso-M. et al. 1992; Cullota 1992; Anderson and Brower 1993, 1996). Numerous lines of research, including the findings of this chapter, thus compellingly argue that logging should not be permitted in the core areas.

Table 4.1. Frequency of occurrence of the 21 plant species found flowering at the Sierra Chincua monarch butterfly colony in March 1994 in relation to forest openness. Each datum represents the number of times the plant species was recorded in each category. The canopy openness categories were: A) Naturally Closed, B) Partially Closed, C) Partially Opened, D) Naturally Open, E) Open, and F) Artificially Opened. See methods for description of categories.

SPECIES	DEGREE OF FOREST OPENING						Total
	CLOSED			OPENED			
	A	B	C	D	E	F	
Eupatorium glabratum	34	5	8	16	12	25	100
E. aschembornianum	28	0	5	1	4	23	61
Senecio prenanthoides	32	0	3	3	4	9	51
Viola grahami	23	1	0	0	1	4	29
Senecio angulifolius	16	0	5	2	2	12	37
Stevia rhombifolia	16	0	5	6	3	19	49
Senecio barba-johannis	3	2	1	3	2	2	13
Senecio cardiophyllus	2	0	0	1	3	3	9
Drymaria cordata	2	0	0	2	0	0	4
Salvia hirsuta	2	0	0	0	0	2	4
Bidens triplinervia	1	0	0	16	1	1	19
Salvia helianthemifolia	1	0	0	1	1	4	7
Senecio roldana	1	0	0	1	0	0	2
Salvia lavanduloides	1	0	0	0	0	2	3
Cirsium ehrenbergii	1	0	0	0	0	0	1
Geranium liliacium	1	0	0	0	0	0	1
Eupatorium mairetianum	0	1	0	0	0	0	1

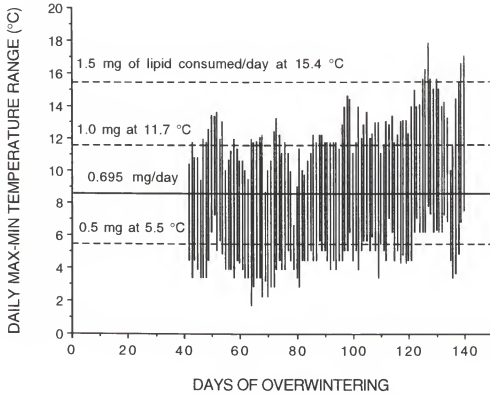
DEGREE OF FOREST OPENING (continued)

<u>SPECIES</u>	<u>CLOSED</u>			<u>OPENED</u>			<u>Total</u>
	<u>A</u>	<u>B</u>	<u>C</u>	<u>D</u>	<u>E</u>	<u>F</u>	
E. pycnocephalum	0	0	0	3	0	0	3
Eupatorium spp.	0	0	0	1	0	0	1
Senecio tolucanus	0	0	0	0	0	3	3
Bacharis conferta	0	0	0	0	0	1	1
Total Stations/category	164	9	27	56	33	110	399^a
Plant species in flower	16	4	6	13	10	14	

^a = One station could not be placed in any of the categories since it had 1/4 naturally open, 1/4 artificially opened and 50% closed canopy.

Figure 4.1. Diurnal range of minimum and maximum ambient temperatures recorded in closed forest at the Sierra Chincua overwintering site. Day 1 refers to November 1, 1993 and day 150 to March 31, 1994. Ambient temperatures were generally stable and progressively became warmer by the end of the season. Dotted lines represent the calculated amounts of lipid mass (mg) consumed per day by inactive monarchs clustered on trees at three different ambient temperatures (Chaplin and Wells 1982). The solid line represents the actual average amount of lipids that clustered monarchs consumed per day during the season (data from chapter 3).

Figure 4.1



CHAPTER 5 BIRD PREDATION ON OVERWINTERING MONARCH BUTTERFLIES: CONSERVATION IMPLICATIONS

Introduction

Each year, the entire population of monarch butterflies (Danaus plexippus L.) from eastern North America spend the winter months on a few mountain peaks of Central México. There, they form tightly packed aggregations of up to 10 million butterflies per hectare (Calvert and Brower 1986). These high concentrations of monarchs are prime targets for vertebrate predators since low ambient temperatures make them largely inactive for the entire 135 day overwintering period (Brower 1985; Masters et al. 1988; Calvert et al. 1989). Furthermore, monarch butterflies are a high quality resource for predators because they accumulate large amounts of lipid reserves during their southward migration (Beall 1948; Brown and Chippendale 1974; Walford 1980; Brower 1985; Calvert and Lawton 1993).

Monarchs, however, possess defensive chemical compounds that are known to be distasteful and toxic to several vertebrate predators (review in Brower 1984; Brower and Fink 1985; but see Petersen 1964; Malcolm 1992). Monarch butterfly larvae feed exclusively on milkweed plants (Asclepiadaceae) from which they sequester toxic cardiac glycosides that are transferred to the adult stage (Brower and Glazier 1975; Nishio 1980; Brower 1984; Malcolm and Brower 1989; Nelson 1993). Adult butterflies

may also obtain pyrrolizidine alkaloids from flowers of the Asteraceae (Kelly et al. 1987). Defensive cardiac glycosides and pyrrolizidine alkaloids appear to limit the number of predator species that feed on monarchs at the overwintering sites. From 37 insectivorous and omnivorous bird species found in the area, only two consistently prey upon monarchs (Fink et al. 1983; Brower and Calvert 1985; Arellano et al. 1993), and only one of five mouse species present at the sites consume monarchs (Glendinning and Brower 1990). Overwintering monarchs are also protected by a group startle response when they detect an attacking predator, a behavior that seems to confuse and disorient bird predators (Tuskes and Brower 1978; Calvert 1994).

Despite these defenses, high rates of predation have been recorded at the overwintering sites in México (Calvert et al. 1979; Brower and Calvert 1985; Glendinning et al. 1988; Arellano et al. 1993), and in California (Tuskes and Brower 1978; Sakai 1994). Brower and Calvert (1985) reported that about 9% of the Sierra Chincua monarch aggregation in México was consumed by black-backed orioles (Icterus galbula abeillei Lesson) and black-headed grosbeaks (Pheucticus melanocephalus Swainson) during the 1978-79 overwintering period. The scansorial black-eared mouse (Peromyscus melanotis J. A. Allen and Chapman) consumed close to 5% of an aggregation that formed at the same site in 1986 (Glendinning et al. 1988).

Monarchs overwintering in México may be particularly vulnerable to predation due to low levels of chemical defense. Malcolm et al. (1993) found that 92% of monarchs overwintering in México fed as larvae on Asclepias syriaca L., a milkweed species that contains cardiac glycosides with low toxicity (Malcolm et al. 1989; Martin et al. 1992). Further, Alonso-M. and

Brower (1994) showed that monarch butterflies become less toxic as the concentration of cardiac glycosides in their bodies decreases with age. Moreover, predators at the overwintering sites feed on monarchs in such a way that they avoid eating the wings and the exocuticle of the thorax and the abdomen, regions where higher concentrations of cardiac glycosides are found (Brower and Glazier 1975; Nishio 1980; Brower et al. 1988; Alonso-M. and Brower 1994).

Monarchs overwinter in forests dominated by the Oyamel fir tree (Abies religiosa H.B.K.). These forests have restricted distributions with high rates of logging and clearing for agricultural fields (Rzedowski 1983; Calvert et al. 1989; Snook 1993). The degradation of the forest where monarchs overwinter endangers their migratory phenomenon (Brower and Malcolm 1991). Five separate overwintering areas in México are protected within the Monarch Butterfly Special Biosphere Reserve (MBSBR). This reserve was divided into buffer zones (10,600 ha), where logging extractions are permitted, and core zones (4, 500 ha), which are areas created for protection of the monarch and all other plant and animal species characteristic of the oyamel fir forest ecosystem (Diario Oficial 1986). Core zones also serve as sources of plant and animal species for the buffer zones.

Current political and local economical pressures are leading government officials to consider the authorization of logging permits in the core areas. In an attempt to link ecological research with conservation strategies for the MBSBR, I studied how monarch butterfly mortality that is caused by bird predators is currently affected by logging extractions that occurred in the 1980s. Brower and Calvert (1985) presented data indicating that the risk of individual monarchs being killed by birds was greater on the periphery of the aggregation and in areas of the forest which had been

thinned. Here, I follow up on their findings by studying bird predation in relation to the degree of openness of the forest canopy. I first designed an objective and practical method to determine closed and open areas in these forests. I then asked if monarch butterflies were preyed upon differentially in closed and open areas.

Methods

Study Site

I monitored butterfly mortality from December 13, 1993 to March 6, 1994 ($n = 83$ days) at the Llano del Toro monarch butterfly aggregation. This 2.01 ha overwintering aggregation formed at an altitude of 3000 m on the southwestern facing slope of the Zapatero Canyon in the Sierra Chincua, a mountain range in northeastern Michoacán, México ($19^{\circ}40'48''\text{N}$ and $100^{\circ}17'54''\text{W}$, Anonymous 1976). This site is one of the principal overwintering areas of the eastern population of monarch butterflies in North America that has been used by monarchs each year since the area was originally discovered in 1975 (Urquhart and Urquhart 1976; Calvert and Brower 1986; Brower 1995a). Large numbers of predatory birds visit the overwintering aggregation in the mornings between 700 and 1000 and in the evenings between 1630 and 1900 hrs. By concentrating their feeding early and late in the day, the birds usually encounter inactive butterflies that are too cold to fly (flight threshold = $9.9 - 16.1^{\circ}\text{C}$, Masters et al. 1988; Alonso-M. et al. 1993). Orioles and grosbeaks are the primary avian predators of monarchs at this and other overwintering sites in México (Calvert et al. 1979).

Determination of Closed and Opened Areas

To determine the degree of forest openness I set six parallel transects, 20 m apart, within the monarch colony. Transects were 10 X 140 m, and were subdivided into 14 contiguous 10 X 10 m quadrats. In each quadrat I estimated (1) tree density as the number of trees/quadrat; (2) total basal area, based on the diameter at breast height for all trees found per quadrat; and (3) forest overstory density, as the percentage of the canopy opened to the sky, based on spherical densiometer readings (Lemmon 1957). Densiometer readings were taken at the corners of all quadrats. At each corner, I moved two meters to the inside of the quadrat and recorded the overstory density four times at the same place, changing the direction of the reading 90° each time. I obtained an average overstory density for each corner, and then estimated an overall average for the quadrat.

Based on these data, I determined that the Llano del Toro monarch overwintering aggregation occupied a portion of forest containing 474 trees/ha, a basal tree area of 49.9 m²/ha, and an average overstory density of 78.9% (S.E. = 0.99; n = 75 quadrats). Nine of the 84 quadrats were excluded in the analysis since they occurred on wide hiking trails or abandoned logging roads.

Tree density, basal area, and overstory density are important variables in determining forest structure (Manzanilla 1974). The maximum datum recorded from each of these variables was used to obtain a relative value per quadrat. I added the three relative values estimated for each quadrat to obtain a quadrat-index value. I then obtained a Closed/Opened Index by estimating the grand average for all quadrat-index values (average = 56.9, S.E. = 1.39, n = 75 quadrats). I classified a quadrat

as closed if its quadrat-index value was above the average of the Closed/Opened Index, or opened if its value was below the average. Using this method I classified 39 of the quadrats as closed and 36 as opened areas. I then randomly selected closed and opened quadrats in which to place nets to capture the remains of monarchs that were actively killed by birds.

At the beginning of the study, I set a total of 25 nets in nine closed quadrats, and 25 nets in nine opened quadrats. Two to three nets were placed per quadrat. Nets were at least four meters apart within the quadrat. As the overwintering season progressed, the monarch aggregation slowly moved down slope to more humid areas. I therefore moved the nets from quadrats that monarchs had abandoned to new quadrats within the aggregation where they regrouped. By January 5, I relocated nine nets (six nets to two new closed quadrats and three nets to one new opened quadrat). Six nets were relocated to three new closed quadrats by January 17, eight nets to three new opened quadrats by February 4, and three nets to one new closed quadrat and five nets to three new opened quadrats by February 14. By the end of the study I had set a total of 40 nets in closed (15 quadrats) and 41 in opened areas (16 quadrats).

Collections of Monarchs Preyed Upon by Birds

In México, birds usually prey upon monarchs on the same branch or tree trunk where they captured the butterflies (Fink and Brower 1981; Brower and Calvert 1985; Arellano et al. 1993). I collected the unconsumed body parts of monarchs as birds dropped them. I used one square meter nets (diameter 1.13 m, depth 0.45 m) suspended 1.5 m above ground to exclude mouse predation (Glendinning et al. 1988; Glendinning and Brower 1990). Daily collections were made at 10 AM when predatory birds

usually ended their morning feast. I collected all items found in the nets and placed them in labeled glassine envelopes. The items found in the nets included complete bodies of dead and moribund monarchs, heads, thoraces, abdomens, dismembered wings, and monarch bodies with zero to four wings attached. I also counted all live monarchs that landed on nets when I tallied monarch mortality.

I modified the method used by Brower and Calvert (1985) and Arellano et al. (1993) to estimate monarch mortality. These authors divided the total number of wings tallied per net by four (the number of wings of a butterfly). Since they did not separate the wings by sex or size, their reports underestimated total mortality. In this study, I determined the sex, size, position and condition of each of the wings found in the nets. With these data I reconstructed individuals from body parts found in each net. For example, if a wing and an abdomen fell into a net I counted them as one predation event unless the wing was from a female and the abdomen was from a male. If I collected two distinguishable wings, two distinguishable abdomens, a thorax with one wing attached of the same size, sex, and wing condition as one of the other two wings, I considered these to be only two predation events despite of all the material recovered. I recorded but did not use in the analyses, butterfly appendages such as antennae, legs, buccal parts, eyes, and small pieces of wings.

Wing size was measured to the nearest 0.5 mm along the costal margin from base to apex. Wing condition was determined subjectively on a scale from one (perfect condition) to five (extremely worn) by looking through the wings into a lamp. Wing condition was based on scale loss, scale color fading, scratches and degree of tattering along the margins (Malcolm et al. 1993; Van Hook 1993).

Effects of Temperature on Bird Predation

To relate bird predation with temperatures, I recorded ambient temperatures with on-site weather logging systems, OWL (Electronically Monitored Ecosystems, 2229 Fifth St., Berkeley, CA 94710). The OWL systems were programmed to register ambient temperatures every minute and to record the average of those measurements over two hour periods. I had one recording station in each of two closed and two opened quadrats. Three temperature probes were placed in the shade at each recording site. Probes were positioned near clustered butterflies at three meters above the ground and were separated by at least five meters. I used the daily minimum and maximum ambient temperatures recorded in closed and opened areas for the analysis.

Estimates of Monarch Density

I estimated the density of butterflies above each net based on the volume that they occupied in a cylindrical area. I projected an imaginary cylinder (diameter two meters, height 25 m) on top of each net ($n = 81$ nets). The percentage of the cylinder that was filled by clustered butterflies was estimated by three different observers. Since estimates did not differ significantly among researchers (Kruskal-Wallis ANOVA, $H = 0.60$, d. f. = 2, $p = 0.96$, data were arcsin transformed, Zar 1984), the median of the three values estimated for each net was used for the analysis.

Comparisons of Clustered vs. Preyed Upon Monarchs

To determine if birds were selectively preying upon certain monarchs, I compared wing size and wing condition of monarchs killed by

birds with inactive monarchs clustered on tree branches. Samples of monarchs were taken from clusters about three to four meters above the ground on December 5, 1993, and on January 10 and February 15, 1994. I measured 50 females and 50 males from each sample, and I used these and all remaining butterflies in the sample to estimate the sex ratio in the cluster.

Statistical Analysis

Daily rates of bird predation for closed and open areas were determined by first calculating the average number of monarchs killed per quadrat per day and then using those data to obtain an overall average rate of bird predation for the two areas. Since the nets were one square meter, results could be analyzed as the number of dead monarchs per square meter per day. I used analysis of variance (ANOVA) to test if the average predation rate was different between closed and opened areas. I used G-tests to determine whether nets located in opened quadrats captured more monarchs than those in closed quadrats. For the analysis, I did not include "dwac" monarchs, i.e. those dead without any apparent cause (108 in closed and 146 monarchs in opened quadrats). The dwac category included mortality probably caused by depletion of fat reserves, dehydration, and/or parasitism by protozoans (Brower and Calvert 1985; Leong et al. 1992; Arellano et al. 1993; Brower et al. 1995).

I used multiple regression analysis to determine the effects of collection date and minimum temperature (independent variables) on bird predation rate (response variable) for both the closed and opened quadrats. I also compared the slopes and y-intercepts of the regression lines of sampling date (covariant) against minimum and maximum ambient

temperature (response variables) registered in closed or opened quadrats (nominal variables) by using analysis of covariance (ANCOVA; Zar 1984).

Monarchs killed by birds in closed and opened quadrats, and those collected from clusters had wing lengths that followed a normal distribution (Shapiro-Wilk W test, $p > 0.05$, Zar 1984). I then compared the averages of these three groups with a parametric ANOVA. Since wing condition was coded as a ranked variable, I used a non-parametric Kruskal-Wallis ANOVA to compare the median wing condition of these three groups. Coefficients of determination (r^2 , Zar 1984) were computed to detect possible correlations between wing length and wing condition.

Results

The average number of monarchs killed per square meter per day was significantly higher in opened than in closed quadrats (ANOVA $F(1, 165) = 6.46$, $p < 0.01$; Fig. 5.1; Table 5.1). I also found that nets in opened quadrats intercepted preyed upon butterflies more frequently than nets in closed quadrats (G-test $G = 35.8$, d. f. = 6, $p < 0.001$).

In closed quadrats, multiple regression analysis showed no significant effect of collection date or minimum temperature on the rate of bird predation (ANOVA $F(2, 82) = 1.37$, $p = 0.26$). In contrast, the rate of bird predation in opened quadrats was significantly higher at the end of the season (ANOVA $F(2, 75) = 10.6$, $p < 0.001$; Beta coefficients: date $t = 3.89$, $p < 0.001$; temperature $t = 0.26$, $p = 0.79$). I also found that ambient temperatures increased through time at the same rate in closed and opened areas during the study, although minimum temperatures registered in

closed areas were consistently warmer during the night than those in opened areas (ANCOVA $F(1, 156) = 58.6, p < 0.001$). Maximum ambient temperatures recorded in the shade during the day did not differ between closed and opened areas in the rate of change through time nor in their y-intercepts (ANCOVA $F(1, 155) = 2.23, p = 0.14$).

There were no differences in wing size (ANOVA $F(2, 165) = 1.5, p = 0.22$), or wing condition (Kruskal-Wallis $H = 1.89, d. f. = 2, p = 0.39$) between monarch butterflies killed by birds in closed quadrats, or in opened quadrats, or live inactive monarchs collected from trees. I did find, however, a significant negative correlation between wing length and wing condition ($r^2 = 0.85, p < 0.001$). Larger butterflies had wings in better condition than did small butterflies.

More male monarchs were killed than females when compared to the sex ratio of live monarchs collected from clusters (G-test = 69.3, d. f. = 1, $p < 0.001$; Table 5.2). I also found a significantly higher number of live, uninjured butterflies landing in nets placed in opened than in closed quadrats (Chi-square = 4.88, d. f. = 1, $p < 0.05$; Table 5.2). The rate of bird predation was not related to the estimated density of monarchs above each net (ANOVA $F(1, 80) = 2.76, p = 0.10$, analysis done in arcsin transformed data).

Discussion

Bird Predation in Closed and Opened Areas

In this study, I showed that monarchs clustered in opened areas were more susceptible to bird predation than those found in areas of closed

forest. Monarch butterflies that overwinter in areas with low tree density, low basal area, and low canopy coverage suffered higher rates of bird predation ($1.308 \text{ m}^2/\text{day}$, S.E. = 0.09) than monarchs in closed areas ($0.994 \text{ m}^2/\text{day}$, S.E. = 0.08; Fig. 5.1, Table 5.1). For overwintering monarchs, as for other species of butterflies and moths, bird predation is a selective agent that affects butterfly behavior and population structure (Collins and Watson 1983; Gibson and Mani 1984; Bowers et al. 1985; Lederhouse 1987). In the overwintering aggregations, bird predation may be one of the forces that have led monarchs to seek out perching sites in areas of closed forest where mortality is lower. During this study, about 20 million butterflies were aggregated in less than 1000 trees. At such a high density, preferred sites may fill in, forcing many monarchs to perch in more opened areas with higher risks of mortality. Opening more areas by logging could severely impact the survival of monarchs overwintering in México.

Further studies are needed to explain why birds attacked more monarchs in opened areas. It could be that in opened areas (1) monarchs are more easily located and captured by predatory birds, (2) individual birds eat more monarchs due to higher energy requirements at colder temperatures, (3) larger flocks of birds feed there, and/or (4) monarch densities are higher. However, I did not find a significant effect of butterfly density on bird predation, discarding this alternative hypothesis. Another possibility is that (5) birds may need to eat more butterflies in opened areas in order to obtain sufficient energy. I found that monarchs in opened areas were more active than monarchs in closed forest (Table 5.2), since monarchs in opened areas are exposed to direct sunlight during the day. This may have caused them to use up their stored fat and therefore to carry lower amounts of lipid reserves than monarchs in closed areas (Chaplin

and Wells 1982; Masters et al. 1988; Chapter 2). Predatory birds may thus need to kill more monarchs for the same amount of food, since they may obtain less lipid mass per butterfly.

The rate of bird predation increased through time in opened areas. I propose four possible but not mutually exclusive explanations. First, similar to the argument discussed above, predatory birds may need to eat more monarchs to fulfill their energy requirements as the overwintering season progresses since monarchs consume their lipid reserves throughout the period. When monarchs arrive to the overwintering sites in November, they have an average of 133 mg of lipid mass (Brower 1985; Masters et al. 1988; Chapter 3). I found that monarchs consume their lipid reserves in relation to the ambient temperatures, such that by the middle of March, just prior to departure from the overwintering sites, they had an average of 56 mg of lipids, a 57.9% decline in five months (see Chapter 3). A second possible explanation is that the concentration of the defensive cardiac glycosides found in monarchs decreases as the butterflies age (Alonso-M. and Brower 1994). Since monarchs are at least 5 months old at the end of the overwintering period, predatory birds may be able to consume more butterfly material as their chemical protection declines. A third hypothesis is that the energy requirements of predatory birds may increase at the end of the season. Birds may require more calories as they prepare for the reproductive events of the spring. Finally, it is possible that the density of birds that attack monarchs at the overwintering aggregations may increase at the end of the season. Further field studies are needed to test these hypotheses.

Predatory birds killed a significantly higher number of male monarchs. The rather low percentage of female monarchs killed by birds

(30.6%) compared to the percentage of live females in collections from either nets or clusters (47.7%), indicates a strong bias towards the consumption of males (Table 5.2). Fink (1980) and Arellano et al. (1993) suggested that grosbeaks prefer to feed on male monarchs, since they have lower amounts of cardiac glycosides (Brower and Glazier 1975; Fink and Brower 1981; Brower et al. 1988; Alonso-M. and Brower 1994). Since orioles seem to consume both sexes equally (Fink and Brower 1981; Brower and Calvert 1985; Arellano et al. 1993), I hypothesize that larger flocks of grosbeaks attacked monarchs during the 1993-94 season, and/or grosbeaks consumed more monarchs per unit of time than did orioles (Fink 1980).

Effects of Temperature on Bird Predation

Brower and Calvert (1985), and Arellano et al. (1993) reported that monarch mortality caused by birds was greater on colder days. In this study, however, I did not find an increase in bird predation on days with lower ambient temperatures. To explore the difference between this and previous studies, I compared the average of the daily minimum ambient temperatures between 16 January to 28 February recorded in this study in closed areas, to the same period in 1979 (from Table 1 in Brower and Calvert 1985) and 1986 (from Figure 3 in Arellano et al. 1993). Since I found overall significant differences between the studies (ANOVA $F(2, 127) = 35.1$, $p < 0.001$), I then performed stepwise unplanned multiple comparisons and found significant differences between all three studies (Ryan's Q test, $p < 0.01$ for all comparisons; Day and Quinn 1989). Coldest temperatures were recorded in 1986 (average minimum temperature = 2.9°C , S.E. = 0.2, $n = 44$ days), warmer temperatures in 1979 (average = 4.2°C , S.E. = 0.2), and even warmer in 1994 (average = 4.8°C , S.E. = 0.1). The present 1994 study may

not have had enough cold days to register high rates of bird predation. Brower and Calvert's 1979 (1985) bird mortality record of 9%, and the 15.5% monarch mortality estimated in this study (see below), may have been higher if the winter had been colder.

Warmer ambient temperatures may influence the feeding behaviors of predatory birds. In a cold year, Arellano et al. (1993) found that orioles, but not grosbeaks, fed cyclically on overwintering monarchs. They reported a 4 day feeding cycle and hypothesized that orioles seemed to accumulate cardiac glycosides as they fed on monarchs and must periodically reduce monarch intake to rid their bodies of these toxins. In a warmer year, Brower and Calvert (1985) found a 7.9 day cycle. In the current study, the warmest of the three, I also used spectral analysis but did not find a feeding cycle in relation to temperature (Priestly 1981; SAS/ETS 1985).

Estimates of Monarch Mortality Due to Bird Predation

I can not directly compare rates of bird predation between the three studies since different methods were used to estimate them. Brower and Calvert (1985) computed daily mortality by dividing the total number of wings found in all nets by 4 (number of wings in a monarch), and by dividing that value by the total number of nets used in a day. They estimated that in one 2.25 ha aggregation, birds killed 2.03 million butterflies, or about 9% of the winter aggregation (density = 10 million monarchs/ha; Calvert and Brower 1986). In the study by Arellano et al. (1993), daily predation rates only included monarchs with damage patterns clearly attributable to orioles or grosbeaks. They did not consider body parts, nor monarchs with unidentifiable damages.

In this study, the reconstruction of individuals based on body parts of preyed upon monarchs allowed me to estimate that birds killed approximately 3.12 million monarchs, or about 15.5% of the winter aggregation. Total mortality was derived by multiplying the average rate of bird predation/m²/day for the entire colony (1.151) X duration of the overwintering period (135 days) X area occupied by the monarch aggregation (20,100 m²). Clearly, monarch mortality was underestimated in previous studies.

Oriole and Grosbeak Predation

I attempted to distinguish butterfly damage caused by orioles and grosbeaks, and asked if these birds preferred to feed in closed or opened areas. As in Brower and Calvert (1985), and Arellano et al. (1993), I followed the descriptions of Fink (1980), and Brower et al. (1988) to identify specific damage to monarchs by either orioles or grosbeaks. I classified monarchs attacked by orioles as those that had a longitudinal slit on their abdominal cuticle. Monarchs attacked by grosbeaks were scored as those without their abdomens. As I conducted the study, I found many dead monarchs trapped in the nets with their abdomen squeezed, damage that is due to both bird species (Fink 1980). I also found many disembodied abdomens with specific damage by orioles. Thus, I did not consider it appropriate to classify monarchs without abdomens as attacked by grosbeaks since orioles could also have caused similar damage. Therefore, I did not compare predation rates between the two bird species. Feeding experiments with caged birds could help resolve this issue.

Implications for Management of the MBSBR

Increased levels of bird predation are not the only detrimental effect that logging may have on the survival of monarch butterflies overwintering in México. Several studies have shown that clustered monarchs freeze to death more often in thinned forests than monarchs in closed canopy forests, especially when winter storms reach the overwintering sites (Calvert and Brower 1981; Calvert et al. 1982; Calvert et al. 1983; Cullota 1992; Anderson and Brower 1996; E. Rendón-S. unpublished data). In addition, monarchs in opened areas are exposed to more sunlight during the day. This results in higher activity levels which causes monarchs to consume their lipid reserves before their return migration to the southern United States (Chaplin and Wells 1982; Masters et al. 1988; Brower and Malcolm 1991; Chapter 2). The Chivati-Huacal monarch aggregation of the MBSBR has become smaller, unstable, and in several years, absent. This is likely due to the high degree of forest openness that has resulted from logging practices (E. Rendón-S., unpublished data). Similar observations have been recorded from degraded forest groves where monarchs overwinter in California (Weiss et al. 1991).

In conclusion, this and previous studies have shown that bird predation on monarch butterflies overwintering in México is a natural event. If the frequency and extent of opened areas in the core zones of the reserve were to decrease (i.e. through seedling regeneration and prevention of illegal logging), bird predation would likely concentrate more at the periphery of the aggregation, as suggested by Brower and Calvert (1985). Predatory birds would also likely find alternative sources of food (Fink et al. 1983). However, by opening areas of forest in the core zones of the MBSBR,

an increased rate of bird predation and freezing mortality would occur. I may also see changes in the clustering preferences by monarchs and in the predatory behaviors of the orioles and grosbeaks. It is clear that more studies are needed to better understand the interactions between the structure of the Oyamel Fir forest and the survival of overwintering monarch butterflies in México. Until then, all current evidence supports the contention that logging permits should not be authorized by the government in the already disturbed core areas of the MBSBR.

Table 5.1. Comparisons of bird predation in closed and open areas for monarch butterflies overwintering at Sierra Chincua, Michoacán, México. Numbers in parenthesis indicate standard errors. Total estimated mortality was derived by multiplying the average rate of bird predation/m²/day X duration of the overwintering period (135 days) X total area occupied by the monarch aggregation (20,100 m²).

	<u>CLOSED</u>	<u>OPENED</u>
Average # of monarchs killed/m ² /day	0.994 (0.08)	1.308 (0.09)
Minimum # of monarchs killed/m ² /day ^a	0.14	0.25
Maximum # of monarchs killed/m ² /day ^b	4.41	4.08
Total # of days sampled	83	83
Estimated number of monarchs killed	2.70 (x 10 ⁶)	3.55 (x 10 ⁶)
Average minimum temperature (°C)	4.7 (0.12)	3.5 (0.12)
Average maximum temperature (°C)	11.8 (0.19)	11.9 (0.18)
Average monarch wing size (mm)	52.5 (0.07)	52.4 (0.05)
Median monarch wing condition ^c	3	3

^a = On December 29th in closed, and on December 27th in opened areas.

^b = By February 13 in both areas.

^c = Scale of 1 to 5 (1 = perfect condition; 5 = extremely worn).

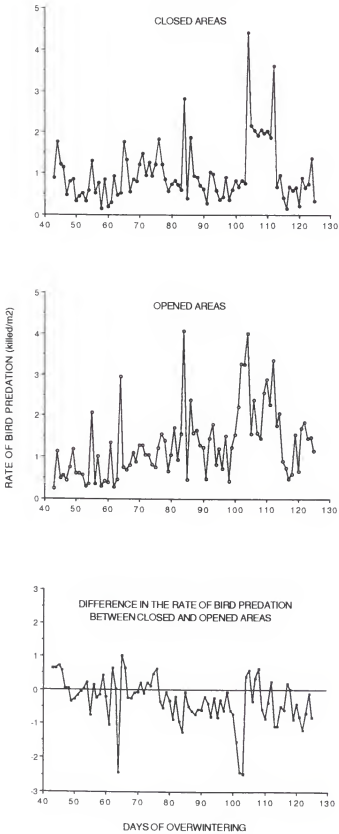
Table 5.2. Sex ratios of female and male monarchs in closed and opened areas at the Sierra Chincua monarch butterfly overwintering site, Michoacán, México, during the 1993-1994 season. Monarchs preyed upon by birds were collected with horizontal nets. Live monarchs were found landing on the nets when I tallied bird predation. Monarchs from clusters were collected from trees at three different times during the season. Males were killed more often than females in both closed and open areas. A higher number of both live and preyed upon monarchs were found in the opened areas.

	<u>FEMALES</u>	<u>MALES</u>	<u>% FEMALES</u>
<u>Total monarchs killed in nets^a</u>			
Closed areas	541	1271	29.9
Opened areas	837	1614	34.2
<u>Total monarchs alive on nets</u>			
Closed areas	3302	3739	46.9
Opened areas	5559	5884	48.6
<u>Monarchs from clusters</u>	297	435	40.6

^a The remains of 234 monarchs were too damaged to be sexed.

Figure 5.1. Comparisons of daily rates of bird predation (average number of monarch butterflies killed/m²/day) in closed and opened areas at the Llano del Toro monarch butterfly aggregation during the 1993-1994 overwintering season. Day 40 refers to December 10 and day 130 refers to March 11, 1994. Differences between closed and opened daily predation rates were calculated by subtracting rates in opened quadrats from those recorded in closed quadrats. Values below zero indicate higher bird predation in opened areas.

Figure 5.1



CHAPTER 6 MECHANISMS OF CARDIAC GLYCOSIDE LOSS AS MONARCH BUTTERFLIES AGE

Introduction

Monarch butterfly larvae (Danaus plexippus L.) sequester cardiac glycosides (CGs) from a variety of milkweed plants (Asclepias spp.). These bitter and emetic compounds are selectively stored and conserved throughout metamorphosis (Reichstein et al. 1968; Roeske et al. 1976; Seiber et al. 1980, 1986; Brower et al. 1982; Lynch and Martin 1987; Martin and Lynch 1988; Malcolm 1990; Malcolm and Brower 1989; Malcolm et al. 1989; Groenveld et al. 1990; Frick and Wink 1995). CGs in adult monarchs are primarily stored in the exoskeleton (i.e. the wings and the cuticle, Brower and Glazier 1975; Seiber et al. 1986; Brower et al. 1988) which provide a chemical defense barrier against natural predation by birds (Fink and Brower 1981; Fink et al. 1983; Brower et al. 1988) and mice (Glendinning et al. 1988; Glendinning 1990). However, the concentration of CGs decreases as adult monarch butterflies age (Alonso-M. and Brower 1994), which may be the reason for the high rates of predation that have been reported on monarchs that range in age from three to seven months during their overwintering phase in México (Brower and Calvert 1985, Arellano et al. 1993; Chapter 5).

Cardiac glycoside loss in monarch butterflies was first proposed by Tuskes and Brower (1978). They studied monarchs overwintering in California and noted that the overall concentration of CGs decreased throughout the period. They proposed that CGs were either metabolized, as adult monarchs do with sugars and lipids, or excreted. Dixon et al. (1978) found that female monarchs that had laid eggs were less emetic to pigeons than freshly eclosed females. These authors and Cohen (1985) proposed that female monarchs lose CGs by passing them into their eggs, and Brower (1984) reported that the mean amount of CGs per egg from monarchs raised on Asclepias curassavica L. was about one μg . Nishio (1980) showed that the meconium, the substance containing the waste products accumulated during the pupal stage, has high concentrations of CGs, implying that monarch butterflies excrete CGs early in the adult stage. In addition, Malcolm and Brower (1989), and Malcolm et al. (1989) proposed that monarchs may lose substantial amounts of CGs during migration since monarchs overwintering in México had lower CG concentrations than butterflies collected in Massachusetts and New Jersey that fed on the same food plant species.

In a laboratory experiment in which adult monarchs were not allowed to mate, Alonso-M. and Brower (1994) found that the concentration of CGs in monarchs decreased as they aged, regardless of both the initial concentration or the CG types sequestered from two different Asclepias spp. CG loss was significant from the wings and from the abdomen, but not from the thorax. Moreover, they also showed that all types of CGs found in adult monarchs are lost in proportion to their initial concentrations and that there was no obvious qualitative change in CGs through time. Therefore, there appeared to be no change in the emetic toxicity of the CGs

as monarchs age, but a progressive decline in the emetic dosage. They proposed four possible mechanisms for this age-related decline in CG content, including CG excretion, denaturation, physical loss of scales from the wings and abdomen, and/or an increased molecular binding of CGs to the cuticle over time.

In this chapter, I conducted experiments to test three possible mechanisms of CG loss. I first determined that CGs were found in adult monarch feces and predicted that their concentration would decrease as monarchs aged. In a second experiment, I tested if the CG concentration decreased through time when monarch butterfly wings were exposed to direct sunlight and daily ambient temperatures. I also measured the rate of scale loss in the wings of reared monarchs, and tested whether the decrease in CG concentration was due to the loss of scales. I hypothesized that older monarchs would have fewer scales on their wings than recently emerged monarchs, and that this would correlate with lower amounts of CGs.

Methods

Experiment 1. Study of CGs in Monarch Feces

I collected feces from monarch butterflies that were three to seven months old. These monarchs were overwintering at Sierra Chincua, Michoacan, México, one of the principal winter areas of the eastern population of the monarch butterfly (Calvert and Brower 1986). Monarchs were netted from clusters two to three meters above the ground. As each butterfly crawled out of the net, it was held by the thorax and the abdomen,

and its wings were pushed up to expose the tip of the abdomen. Most butterflies excreted feces while struggling to escape. Each fecal sample was collected by holding the butterfly above a three milliliter glass vial, being careful not to touch the vial with the body of the butterfly, thereby avoiding contaminating the sample with scales, since they contain CGs (Nishio 1980). Female and male samples were pooled in different vials and analyzed separately.

Fecal samples were collected during two overwintering seasons. On December 21, 1993, and January 18, 1994, I collected and pooled fecal samples from 5, 10, 15, 20, 25 and 50 female monarchs and placed them in separate numbered vials with 1 ml of ethanol (six replicates for each of the pooled samples). Male feces were collected in the same manner. I used this sampling procedure to determine the number of fecal samples needed to detect CG concentrations by a standard spectrophotometry assay described in Malcolm et al. (1989). On February 13 and March 17, 1994, I collected eight samples with a 100 female monarch feces per vial, and eight samples with a 100 male monarch feces per vial. All vials were stored at -4°C until CG analysis was performed. In the summer of 1994, I determined that 50 pooled fecal samples produced detectable amounts of CGs. I therefore pooled the samples from December 1993 to have 50 feces per vial. For example, 2 vial with 15 monarch feces, and one with 20 were combined; two vials with 25 monarch feces were also combined. The ethanol was then evaporated from the combined samples and the vial was brought back to one milliliter volume. I followed the same procedure for the January 1994 sample, resulting in eight replicates with 50 monarch feces samples for each sex in December and January. The February and March samples from 100 butterflies were diluted into two ml of ethanol to obtain

the appropriate concentration of 50 samples per vial. During the 1994-1995 overwintering season in the Sierra Chincua, I collected 50 feces per vial on January 8 (seven vials per sex), February 8 (eight vials per sex), and March 9 (nine vials per sex).

Cardiac glycoside concentrations were determined as $\mu\text{g}/0.1\text{g}$ dry weight equivalent to digitoxin, using the spectrophotometric assay described in Malcolm et al. (1989). I then confirmed the presence of CGs in the fecal samples by performing thin-layer chromatography (TLC) on Merck Silica gel 60 F₂₅₄ plates. Plates were developed four times in a chloroform-methanol-formamide (90:6:1) solvent system, as described in Brower et al. (1982) and Malcolm et al. (1989). In two previous studies, Seiber et al. (1986) and Malcolm et al. (1989) found that most monarchs overwintering in México fed as larvae on Asclepias syriaca L., a milkweed species found in the northern United States. I thus expected the TLC patterns to be similar to that of A. syriaca.

Experiment 2. CG Denaturation in Monarch Wings

Five monarch females were collected in Miami, Dade County, Florida, on October 25, 1995. Twelve eggs from each monarch were reared in the laboratory at approximately 25°C under ambient and fluorescent ceiling light. Larvae fed on Asclepias curassavica L., a milkweed food plant with high concentration of CGs (Brower 1984). Adults emerged November 22 and 23, 1995. One day after emergence, I measured the length of the right fore wing (mm) and killed all adults once they had released their meconium and their wings had hardened. To avoid tissue decomposition, I removed the four wings from the body of the butterfly. I only analyzed the wings because Alonso-M. and Brower (1994) showed that

the concentration of CGs decreased at the same rate in the wings and abdomen. Since they did not find significant differences between females and males (Alonso-M. and Brower 1994), butterfly sex was not considered a factor in the current study.

Thirty randomly selected individuals (i.e. 30 sets of 4 wings) were kept in a freezer at -4°C and the other 30 were pinned onto the surface of wooden butterfly mount boards with entomological pins. I placed the mounting boards in a greenhouse with the upper side of the wings exposed to direct sunlight and daily temperature fluctuations ranging from 7 to 32°C. At 1, 3, 5, 9, 17 and 29 days after emergence, I randomly selected wings from five individuals from the greenhouse and from the freezer. On those dates, the wings were dried at 60°C for three hours and weighed. Fat was then extracted from the wings as described in Walford (1980) and May (1992), and CG analysis was performed as described above.

Experiment 3. Scale Loss in the Wings of Reared Monarchs

Alonso-M. and Brower (1994) aged monarchs reared on Asclepias humistrata Walt. in a screened outdoor flight cage for up to 25 days in Gainesville, Florida, and systematically collected butterflies of different ages. Prior to analyzing the wings of the adult butterflies to determine CG concentration, they removed 0.6 cm diameter disks from the discal cell of the left hind wing using a sharp cork borer. Each sample was mounted and labeled between two microscope slides, and saved until analyzed in the present study. With the aid of an Olympus stereoscopic microscope, I counted the number of missing scales on the upper side of the wing disk in an area of 3200 scales (40 rows X 80 columns of scales). Without reading the label, I focused the wing disk at the center of the field of view. Each disk

had about 100 scales at the equator. I counted the number of missing scales from left to right on the field of view. I did not count the first 10 columns of scales on either side, since the ones closer to the edge were removed when the disk was cut. I thus counted the number of missing scales from a total of 80 scales at the equator, and the ones missing from 20 rows above and 19 rows below the equator.

Statistical Analysis

Once I found that fecal samples of adult monarchs had detectable concentrations of CGs, I performed a two way analyses of variance (ANOVA) to test if the concentration of CGs was significantly different through time, and if there were differences between the two sampling years (Zar 1984). In the second experiment, I used multiple regression analysis to detect significant effects of experimental date, dry weight, and wing length (independent variables) on CG concentration (response variable) of wings kept in the freezer and wings exposed in a greenhouse. I used simple regression analysis to estimate the rate of scale loss in the wings of aging monarchs. I also regressed the number of missing scales in the wings against CG concentration. If I found a significant effect on the dependent variable, logarithmic, exponential, and inverse transformations were performed. Models with the highest coefficient of determination (r^2) were selected.

Results

Experiment 1. Study of CGs in Monarch Feces

I found that monarch fecal samples had detectable amounts of CGs and that CG concentration significantly decreased as monarchs aged (ANOVA $F_{(3, 95)} = 9.03$, $p < 0.001$; Fig. 6.1). No difference in the CG concentration was found between years (ANOVA $F_{(1, 95)} = 0.17$, $p = 0.68$). The CG patterns found in the TLC analysis were virtually identical to those found by Malcolm et al. (1989), indicating that the overwintering monarchs had fed as larvae on A. syriaca. I estimated that CG concentration found in monarch feces decreased from 70 to 40 $\mu\text{g}/0.1\text{g}$ dry weight in 87 days during the 1993-94 overwintering colony (Fig. 6.1).

Experiment 2. CG Denaturation in Monarch Wings

Multiple regression analysis showed that age, dry weight, and wing length had no significant effects on the CG concentration in monarch wings stored in a freezer at -4°C (ANOVA $F_{(3, 34)} = 0.47$, $p = 0.71$; Fig. 6.2). However, the same analysis on monarch wings exposed in the greenhouse showed that experimental date had a significant effect on CG concentration (ANOVA $F_{(3, 34)} = 7.87$, $p < 0.001$; Beta coefficient: date $t = 2.61$, $p < 0.01$), while dry weight ($t = 1.95$, $p = 0.06$), and wing length ($t = 0.90$, $p = 0.37$) had no significant effects. I used analysis of covariance to compare the rate of CG loss between the two treatments and found a highly significant difference (ANCOVA $F_{(1, 56)} = 10.7$, $p < 0.001$). I estimated a mean loss of 200 $\mu\text{g}/0.1\text{g}$ dry weight of CGs over the 29 days that the wings were exposed in the greenhouse (Fig. 6.2).

Experiment 3. Scale Loss in the Wings of Reared Monarchs

Simple regression analysis showed that living monarch butterflies aged over 25 days lose scales from their hind wings with time (ANOVA $F_{(1, 70)} = 102$, $p < 0.001$; Fig. 6.3). I also found a significant negative exponential relation between the number of missing scales in the monarch wings and the CG concentration (ANOVA $F_{(1, 70)} = 53.2$, $p < 0.001$; Fig. 6.4). Thus, as monarchs lost scales, they also lost about 300 $\mu\text{g}/0.1\text{g}$ dry weight of CGs.

Discussion

Cardiac glycosides sequestered and stored during larval feeding impart to the adult butterfly a degree of unpalatability that is dependant both upon CG type and concentration (Alonso-M and Brower 1994). Unlike chrysomelid beetles (Pasteels and Daloze 1977; Van Oycke et al. 1987), monarchs do not have metabolic pathways that synthesize CGs (Nelson 1993). Alonso-M and Brower (1994) previously determined that the concentration of CG decreased closed to 600 $\mu\text{g}/0.1\text{g}$ dry weight as the butterflies aged 25 days. Therefore, recently emerged butterflies with high concentrations of CGs will be better protected against predation than older monarchs. In this study, I demonstrated three mechanisms by which monarchs lose CGs: by losing scales from 500 to 200 $\mu\text{g}/0.1\text{g}$ dry weight (25 days), by denaturation of the CGs in their wings from 650 to 450 $\mu\text{g}/0.1\text{g}$ dry weight (29 days), and by excretion in fecal matter from 70 to 40 $\mu\text{g}/0.1\text{g}$ dry weight (87 days). The combined effect of these three mechanisms accounted for about 530 $\mu\text{g}/0.1\text{g}$ dry weight of the CG loss. Other mechanisms such as CG catabolism may also occur.

Scale loss seems to be the most important factor explaining CG decrease in adult monarch butterflies, since it accounted for 300 $\mu\text{g}/0.1\text{g}$ dry weight, about half of the total loss estimated by Alonso-M. and Brower (1994). Nishio (1980) showed that wings without scales had lower concentrations of CGs and weaker TLC patterns compared to complete wings. Data from this study showed that CG loss was highest for older monarchs, which had considerably fewer scales on their wings than did recently emerged monarchs (Figs. 6.3 and 6.4). Monarch scales have high concentrations of CGs (Nishio 1980; Brower et al. 1988), and they are not replaced once they are removed from the body of the butterfly (Urquhart 1987).

In the greenhouse experiment, I showed that the CGs of monarch wings exposed to sunlight and daily ambient temperatures had lower concentrations than monarch wings stored in a freezer (Fig. 6.2). I eliminated the possibility that the observed CG decrease was due to scale loss by comparing the number of missing scales in the hind wing of individuals from both treatments (greenhouse and freezer) collected during the last two experimental dates (days 17 and 29). I found no significant differences between the two treatments in the number of missing scales (Student t-test = 0.76, d. f. = 18, $p = 0.46$). Denaturation of CGs may thus have occurred due to exposure to ultraviolet light and the ambient weather conditions present in the greenhouse (loss of about 200 $\mu\text{g}/0.1\text{g}$ dry weight; Fig. 6.2). Although CGs are stable compounds (Malcolm 1991; Nelson 1993), Reichstein (1968) found that CGs that contain an aldehyde group (e.g., calotropin) are sensitive to auto-oxidation. Since the quality of CG does not change in adult monarchs of different ages (as determined by the position of TLC spots, Alonso-M. and Brower 1994), the different types of

CGs present in adult monarchs must be equally altered. Additional evidence that CGs may denature comes from the fecal samples collected from overwintering monarchs. The concentration of CGs detected in monarch feces also decreased through time (Fig. 6.1). An alternative explanation for the observed CG decrease in fecal samples is that older monarchs may control the amount of CGs that they excrete. This hypothesis can be tested by tracing radioactively marked CGs throughout the life of a monarch.

Catabolism of CGs may also occur in adult monarchs. Metabolic alteration of CGs has been shown to occur in monarch larvae (Nishio 1980; Seiber et al. 1980; Marty and Krieger 1984; Malcolm et al. 1989; Nelson 1993). Monarch larvae metabolized nonpolar CGs (e.g. uscharidin, labriformin) to CGs of intermediate polarity and conjugated aglycones (e.g. calactin, calotropin, aspecioside, digitoxigenin; Nelson, 1993). Insects cannot synthesize cholesterol, the source of non-sequestered steroid metabolites in animals. Herbivorous insects require phytosterols (i.e. substrates for steroids such as CGs) to synthesize the sterols they need (e.g. for production of juvenile hormone; Kircher 1982; Wigglesworth 1983). Monarchs, thus may hydrolyze the sugar and the butenodiol molecules from the CG, and use the steroid ring for cholesterol, although sugars with double linkage (C2 - C3) make the CG very resistant to acid hydrolysis (Brown et al. 1979).

An additional component to the lowering of CG concentration through time could be due to an increased binding of CGs to the exocuticle. Predators of insects usually do not completely break down insect cuticular parts and their remains are often intact in the predator feces (Fink et al. 1983; Redford 1984). So, if the extraction technique (i.e. water bath at 78°C in ethanol for one hour) was insufficient to dissolve CGs that become

progressively more strongly, covalent-bonded to the cuticle, the physical and chemical properties of CGs would change as a result from the binding such that the new compounds would unlikely produce either an emetic effect on monarch predators nor a reaction to the calorimetric agent (C. Nelson, personal communication).

In conclusion, I have shown that a combination of three mechanisms, including scale loss, denaturation, and excretion, can account for perhaps the entire decrease in CG concentration during a monarch lifespan (from 800 to 200 $\mu\text{g}/0.1\text{g}$ dry weight, Alonso-M. and Brower 1994). Since the decrease in CG concentration results in monarchs suffering emetic deterioration as they age (Alonso-M. and Brower 1994), predatory birds may use visual cues to select older butterflies (MacLean et al. 1989), since they do not have their wings as brightly colored as recently emerged individuals. However, adult monarch butterflies actively sequester and store pyrrolizidine alkaloids (PAs) from several plant families, particularly Asteraceae (Boppre 1986; Kelley et al. 1987). Storage of these compounds by adult danaid butterflies may also have a defensive function (Ackery and Vane-Wright 1984). This may mean that as CGs decrease, the concentration of PAs could increase as the butterflies age. Further studies need to address the interactions between CGs and PAs in the chemical defense of monarch butterflies.

Figure 6.1. Cardiac glycoside concentration in monarch butterflies feces. Samples from monarchs overwintering in México during the 1993-94 season. Day 40 refers to December 10, and day 140 to March 20. Monarch lost close to 30 $\mu\text{g}/0.1\text{g}$ dry weight during the study period.

Figure 6.1

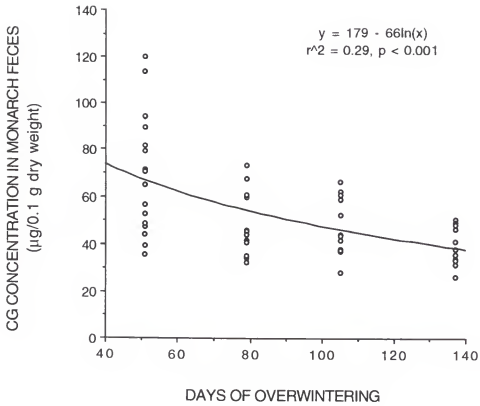


Figure 6.2. Cardiac glycoside concentration in monarch wings under two experimental treatments as a function of time. The concentration of cardiac glycosides of monarch wings stored at -4°C did not change whereas the concentration of monarch wings exposed in a greenhouse decreased through time. The average loss of CGs over the 30 day period was $200\text{ }\mu\text{g}/0.1\text{g}$ dry weight.

Figure 6.2

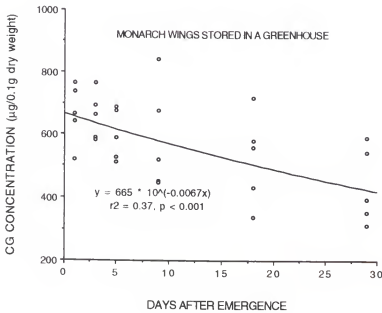
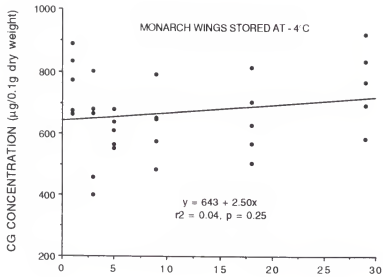


Figure 6.3. Relationship of the number of wing scales that adult monarch butterflies lose while aging in a outdoor flight cage in Gainesville, Florida. The older the butterfly, the higher the number of missing scales in their hind wings.

Figure 6.3

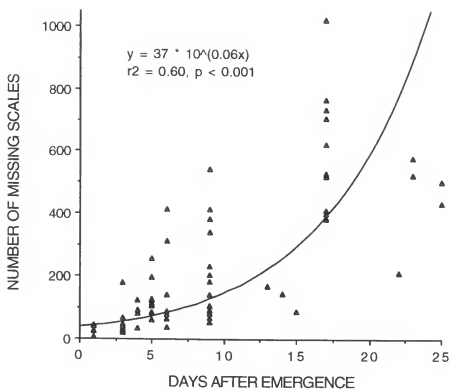
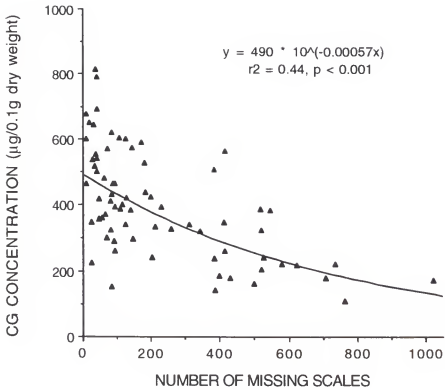


Figure 6.4. Relationship of the number of missing scales from monarch butterflies wings and CG concentration. Monarchs were aged in an outdoor flight cage. As monarch butterflies lose their scales, the concentration of CGs decreases. Scale loss seems to be the most important factor explaining CG decrease in adult monarch butterflies, since it accounted for 300 $\mu\text{g}/0.1\text{g}$ dry weight of CG loss, about half of the total loss estimated by Alonso-M. and Brower (1994).

Figure 6.4



CHAPTER 7

CONCLUSIONS AND FUTURE RESEARCH

We know the name of most of the species
in the forest, but we do not know the
name of the people that obtain their
livelihood from it. (P. Berner 1996, during a seminar)

My dissertation provides several lines of strong evidence that logging is detrimental to the survival of monarch butterflies overwintering in México. Studies conducted at the Sierra Chincua reserve, one of the most pristine sites in the MBSBR, revealed that it has been altered by prior logging activities. Since the core areas of the MBSBR are relatively small (Table 1.1), future logging could severely impact the microclimate and biological balance required by monarchs for successful overwintering. Monarchs need cool temperatures to preserve their lipid reserves (Fig. 4.1), and they seemed to prefer relatively high humidities (Figs. 2.2 and 2.5), possibly to reduce desiccation. Using a series of experiments, I showed that butterflies clustered in areas opened by logging were exposed to colder temperatures during the night (Fig. 2.1) and to higher wind speeds (Fig. 2.3). These factors raise the risk of freezing mortality and increase flight activity to water sources. The pattern of lipid loss observed in monarchs clustered in shaded areas (Figs. 2.6 and 3.4) is consistent with the hypothesis that intact, closed forest, is necessary for successful overwintering because this permits them to conserve their lipid reserves for the spring migration back to the United States. I also found that the core

area of the Sierra Chincua MBSBR already has a substantial number of areas with human-induced disturbances (Table 4.1), that the plants in flower were common during the winter at this site, and that closed and opened areas had the same plant species composition. Therefore, I recommend no further logging on the core areas of the MBSBR.

As I discussed in the introduction, more studies are needed on the ecology of the Oyamel fir tree. By learning the ecology of seed production, dispersal and germination, the forest could be managed to promote natural regeneration, instead of the expensive reforestation that is so encouraged by politicians and rural developers (Chapela and Barkin 1995). For example, in the last ten years that I have worked on this type of forest, I have observed that seedlings tend to be in clumped distributions. I originally hypothesized that the seedlings are clumped as a result of the dispersion of the seeds, since I have observed that mature cones of Oyamel trees usually fall to the ground before the seed are released. However, at the end of the 1993-94 winter period, I observed that the majority of trees produced large quantities of pollen. As expected, in the spring of 1995, a great number of the winged seeds were liberated and to my surprise, most of them were released while the cones were still on the trees. Given the climatic conditions of light rain and strong winds at the time, thousands of seeds were "uniformly" dispersed on the forest floor (E. Rendón unpublished data). Since the relationship between forest density, understory vegetation, and natural and human disturbance with respect to natural forest regeneration in oyamel fir forests is not well understood, I took this opportunity to test several hypothesis about fir forest regeneration.

In general, forest engineers propose that human disturbance (e.g. logging) is needed for the natural regeneration of these forests and that it

should be supplemented with artificial reforestation (C. Avalos, personal communication). They argue that the understory vegetation, including the carpet of mosses, prevents seedling germination and establishment. In collaboration with Eduardo Rendón and Eneida Montesinos, two students of the Center of Ecology, National University of México, we set a series of experiments to study seed germination, and seedling survival and growth in areas with (1) high and low density of understory vegetation; (2) where the understory vegetation was removed; and (3) where grazing by cattle was prevented (i.e. by using wire enclosures). We will be monitoring the seedling survival and growth on these plots for at least ten years. Clearly, studies such as this can teach us the ecology of the Oyamel forest. Additional experiments to study the effects of fertilizers applied to forest plantations could be useful (Gower et al. 1992).

It is frequently argued that the Oyamel forest has large quantities of trees that are infested with dwarf mistletoe, bark beetles, and/or insect defoliators, and that those trees should be removed to prevent further infestations. However, little is known about the distribution, abundance, or vertical and horizontal transmission of these diseases. Since nothing is known about the natural population control of these diseases, careful consideration should be taken before developing chemical or removal management plans (Snook 1993). The dwarf mistletoe flowers in March-April and fruits are produced in October-November when the sticky seeds disperse and infect neighboring branches (Rodríguez 1983). The dwarf mistletoe can only be detected in adult trees when they produce "witches brooms" or fruiting structures. The mistletoe deforms and reduces growth and seed production in mature trees, killing seedling and saplings and thus affecting natural and artificial regeneration. It also weakens the tree,

making it susceptible to attack by other organisms that could lead to mortality. A detailed survey of the frequency of mistletoe infestation is needed before management decisions can be made. Observations such as the frequency of seedling regeneration in the proximity of infected trees should be made. Laboratory and field experiments should address the effects of mistletoe on seedling survival. Similar studies should be also conducted on the rates of infestations by bark beetles and geometrid defoliators.

According to Snook (1993), forest management in the MBSBR should integrate two main objectives: the maintenance and improvement of forest structure for monarch butterflies, and the development of alternative sources of material goods and income for local people who currently derive much of their livelihood from the fir forests. More studies are needed to accomplish both of these objectives. The exclusion of free range livestock from the fir forests may help the first objective by improving seedling regeneration. However, cattle raising in corrals would require extra efforts by the owners to supplement water and food. The effects of restricting livestock from forests could be tested in reserves owned by the federal government but the results would not be seen for one or two decades. Few researchers, politicians, or local peasants can afford to wait so long.

The second objective can be accomplished by studying the economical and the socio-political status of the 30 ejidos that are affected by the MBSBR. Most of the local people live on income derived from logging and agriculture (Barkin and Chapela 1995). Basic information on the amount, origin and destination of wood taken from the MBSBR by each ejido for domestic and industrial purposes needs to be evaluated. Studies on the access and control of resources by females and males (i.e. gender analysis) at the

family level are also needed. With these data, comparative studies could be conducted to see, for example, the positive or negative effects (e.g. increased income or a positive attitude toward conservation) that tourism has brought to the ejidos that own the forest where monarchs overwinter (Rosario and Cerro Pelón, Ejido Capulin) compared to those that do not have extra income from tourism. Studies of the rates of population growth and emigration to Mexico City would also be useful (Chapela and Barkin 1995).

A landscape approach could be implemented for the re-transformation of agricultural to forested land. Ejidatarios could be encouraged to use those parts of their land that are not productive (due to steep slope or erosion) to implement silvicultural practices (pine plantations) or to implement agricultural techniques that increase the yield of agricultural products. An economic analysis of the product markets could help ejidatarios sell their products to better buyers. This approach would require an endowment to subsidize revenues and encourage families to try it. Pine plantations can be economically important. Pines grow relatively fast and the wood has a good market value. An additional value of establishing forest plantation is that soil erosion is reduced and other animal and plants can exist there.

A famous Mexican poet, Homero Aridjis from a conservationist group known as "the group of the 100", and Dr. Lincoln Brower, from the University of Florida, have recently put forth the idea to buy the land of the MBSBR from the ejidos (The New York Times, 26 January 1996). They argue that by privatizing the reserve, they will eliminate the logging pressures. However, the current political and economical situation is not as simple as just buying the land. First, the ejidatarios of El Rosario recently stipulated that their land is not for sale (La Jornada, 20 February

1996). Second, as it has happened in Costa Rica where the "Monte Verde Conservation League" bought land to preserve cloud forest, peasants that sold their land for large amounts of money often had to go to work for the new owner after a few years of "good life". Payments over time also do not usually work, since most people do not like to have restrictions placed on the use of their money (A. Cruz personal communication, ejidatario of El Rosario). Third, even if the reserve is privatized, the local people will likely continue to extract forest products as they have done for generations. To keep people out, the army would likely need to be brought in. This would make the politics of conservation even more complicated and unpopular with the local people. I am convinced that only through education (environmental, economic, political and medical, e.g. methods of birth control) and by the implementation of wise forest management can the MBSBR be a model for conservation biology.

Most of the alternatives to deforestation presented here will require large amounts of money and much time. These efforts would need to have the input from all the organizations that are, for one reason or another, involved with the conservation issues of the monarch butterfly. These include the federal government of México (Instituto Nacional de Ecología and Procuraduría Federal de Protección al Medio Ambiente), the state governments of Michoacán and México, nine counties (municipios), more than 30 ejidos, national (ProNatura, Group of the 100) and international (Wildlife Conservation and Society, World Wildlife Found, Conservation International) conservation organizations, and national (National University of México, Universidad Autónoma Metropolitana, Universidad Michoacana de San Nicolas de Hidalgo, Colegio de México) and international (University of Florida) research institutions. All of us

involved in this project have the same objective: the preservation of the migratory phenomenon of the monarch butterfly and of the Oyamel fir forests where they rest during the winter. With scientific knowledge as our base, we must now join our efforts to enhance the economic development of local communities which monarchs have visited every year for hundreds of years, so that the monarchs may continue to do so for many years to come.

APPENDIX LIPID AND LEAN CONTENTS IN THE ANNUAL CYCLE OF THE MONARCH BUTTERFLY

Date, location and source of monarch samples assayed for lipid analysis during their annual life cycle. Sample size (N), average lean mass (S.E.), average lipid mass (S.E.), and range of lipid mass are given in milligrams. Lean mass is the dried mass of the butterfly minus the lipid mass. Samples are listed in chronological order in each category.

SITE	DATE	N	LEAN	LIPID	RANGE	REFERENCE
A) <u>FRESHLY ECLOSED</u>						
1) California	1972	25	112a	35a	---	Ch. & Wells 1982
2) Massachusetts	Sep 77	45	179 (3)	29 (2)	5-57	Brower 1985
3) Florida	Jul 81	10	140 (10)	14 (2)	---	Cohen 1985
4) Australia	1982	20	148 (10)	22 (6)	---	James 1984
B) <u>SUMMER BREEDING</u>						
5) Wisconsin	Jun 85	605	142 (1)	12 (1)	2-54	Malcolm et al. ^b
6) Wisconsin	Jun 86	159	145 (3)	20 (1)	4-85	Malcolm et al. ^b
7) Wisconsin	Jun 94	333	148 (2)	10 (1)	3-72	Brower et al. ^b
8) Massachusetts	Jul 79	109	160 (4)	18 (2)	3-56	Walford 1980
9) Massachusetts	Aug 79	174	172 (7)	23 (2)	2-86	Walford 1980
10) Massachusetts	Aug 79	230	161 (2)	19 (1)	---	Brower 1985
11) Ontario	Aug 86	79	185a,c	54a,c	---	Gibo & McC. 1993
12) Australia	Nov 82	12	141 (10)	26 (3)	---	James 1984
13) Australia	Dec 82	22	133 (5)	27 (2)	---	James 1984
14) Australia	Jan 83	19	132 (8)	17 (3)	---	James 1984
15) Australia	Feb 83	20	121 (7)	21 (2)	---	James 1984

APPENDIX--continued

SITE	DATE	N	LEAN	LIPID	RANGE	REFERENCE
<u>C) AUTUMN MIGRATION</u>						
16) Ontario	Sep 40	162	104a,d	55a,d	---	Beall 1948
17) Ontario	Sep 41	122	106a,d	66a,d	---	Beall 1948
18) Ontario	Sep 43	180	107a,d	102a,d	---	Beall 1948
19) Missouri	Sep 72	10	166 (6)	137 (8)	---	Brown & Ch. 1974
20) Massachusetts	Sep 79	137	168 (4)	26 (2)	5-76	Walford 1980
21) New Jersey	Sep 79	50	178 (5)	46 (5)	5-107	Walford 1980
22) Kansas	Sep 79	122	165 (6)	19 (2)	3-96	Walford 1980
23) Ontario	Sep 86	73	184c	79a,c	---	Gibo & McC. 1993
24) Ontario	Sep 86	82	157c	35a,c	---	Gibo & McC. 1993
25) West Virginia	Oct 70	5	--	100a	---	Cenedella 1971
26) Hidalgo, Mex.	Oct 77	101	170 (3)	146 (4)	8-281	Walford 1980
27) Massachusetts	Oct 79	105	172 (3)	24 (2)	6-58	Walford 1980
28) Florida	Oct 79	108	171 (3)	29 (3)	5-143	Walford 1980
29) Texas	Oct 79	42	173 (2)	112 (8)	21-218	Walford 1980
30) Texas	Oct 79	66	179 (6)	100 (6)	5-184	Walford 1980
31) Texas	Oct 82	59	174 (4)	77 (5)	24-226	Brower et al. ^b
32) Texas	Oct 93	132	172 (2)	120 (5)	7-296	This paper
33) Georgia ^e	Nov 80	46	169 (4)	20 (4)	4-54	Brower et al. ^b
34) Florida ^e	Nov 82	120	170 (4)	19 (3)	5-61	Brower et al. ^b
35) Florida ^e	Dec 81	43	134 (5)	14 (3)	---	Cohen 1985
<u>D) OVERWINTERING AGGREGATIONS</u>						
36) California	Nov 71	25	168a	108a	---	Ch. & Wells 1982
37) California	Nov 75	51	189 (7)	67 (6)	---	Tusk. & Bro. 1978 ^f
38) California	Nov 75	104	160 (6)	83 (4)	---	Tusk. & Bro. 1978 ^f
39) México	Nov 82	174	163 (4)	142 (4)	54-227	Brower et al. ^b
40) México	Nov 93	100	170 (2)	133 (4)	20-237	This paper
41) California	Dec 71	25	174a	100a	---	Ch. & Wells 1982
42) California	Dec 75	100	160 (4)	61 (3)	---	Tusk. & Bro. 1978 ^f
43) México	Dec 77	78	150 (4)	143 (4)	27-247	Brower et al. ^b

APPENDIX--continued

SITE	DATE	N	LEAN	LIPID	RANGE	REFERENCE
44) México	Dec 83	30	179 (5)	127 (4)	59-262	Brower et al. ^b
45) México	Dec 93	100	183 (3)	113 (4)	17-258	This paper
46) California	Jan 72	25	157 ^a	63 ^a	---	Ch. & Wells 1982
47) México	Jan 77	101	150 (5)	115 (4)	24-270	Brower et al. ^b
48) México	Jan 78	105	162 (6)	107 (5)	18-223	Walford 1980
49) México	Jan 79	157	160 (2)	82 (4)	4-189	Walford 1980
50) México	Jan 81	99	165 (2)	81 (6)	7-241	Brower & M. 1991
51) México	Jan 83	47	167 (4)	117 (7)	49-195	Brower et al. ^b
52) México	Jan 94	100	172 (2)	101 (4)	13-224	This paper
53) California	Feb 72	25	166 ^a	57 ^a	---	Ch. & Wells 1982
54) California	Feb 76	102	153 (3)	39 (2)	---	Tusk. & Bro. 1978 ^f
55) México	Feb 78	100	155 (4)	69 (4)	6-198	Brower et al. ^b
56) México	Feb 78	100	162 (5)	56 (4)	5-126	Brower et al. ^b
57) México	Feb 83	86	161 (6)	107 (4)	22-227	Brower et al. ^b
58) México	Feb 94	100	164 (2)	58 (4)	6-163	This paper
59) México	Mar 78	101	159 (3)	59 (4)	3-151	Brower 1985
60) México	Mar 94	94	168 (2)	56 (3)	14-149	This paper
61) Australia	Apr 83	71	180 (5)	94 (7)	---	James 1984 ^g
62) Australia	May 83	89	185 (4)	73 (4)	---	James 1984 ^g
63) Australia	Jun 83	69	175 (4)	53 (4)	---	James 1984 ^g

E) FLOWER-VISITING MONARCHS WHILE OVERWINTERING

64) México	Dec 93	100	153 (3)	53 (4)	6-179	This paper
65) México	Jan 81	133	166 (2)	21 (3)	4-158	Brower & M. 1991
66) México	Jan 94	100	153 (2)	24 (3)	6-134	This paper
67) México	Feb 94	100	148 (2)	21 (2)	6-88	This paper
68) México	Mar 94	100	157 (2)	21 (2)	6-111	This paper

F) SPRING BREEDERS IN THE SOUTHERN UNITED STATES

69) Texas-Louisia	Apr 85	129	143 (2)	17 (2)	4-100	Malcolm et al. ^b
70) Texas-Louisia	Apr 86	167	146 (2)	27 (2)	2-112	Malcolm et al. ^b

APPENDIX--continued

SITE	DATE	N	LEAN	LIPID	RANGE	REFERENCE
71) Texas-Oklaho	May 85	116	159 (3)	35 (3)	2-95	Malcolm et al. ^b
72) Texas-Oklaho	May 86	431	156 (2)	29 (1)	4-120	Malcolm et al. ^b

a Standard error not published.

b Unpublished work.

c Median lipid mass reported.

d Lipid analysis excluded the head and the wings.

e Sample of non-overwintering monarchs.

f They used a different method for lipid extraction.

g Combined data from three overwintering areas.

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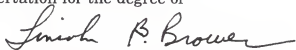
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BIOGRAPHICAL SKETCH

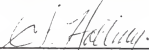
Alfonso Alonso-Mejía was born on 18 June 1963 in México City, México, where he spent most of his childhood. He became immersed in the study of biology while traveling throughout México with his parents. In 1982, he attended the Facultad de Ciencias at the Universidad Nacional Autónoma de México (UNAM) and graduated with a Bachelor of Science degree in biology in September 1987. For his bachelor thesis he spent several months during three years in the overwintering colonies of monarch butterflies, in Michoacán, México. In the fall of 1988, Alfonso began graduate studies in the Department of Zoology at the University of Florida, under the guidance of Dr. Lincoln P. Brower. He received his M. S. degree in May 1991, for work on the effects of aging on the chemical defensive compounds that monarch butterflies sequester from their larval food plants. Upon completion of his Ph. D., he will be moving to Norman, Oklahoma, where his wife, LeeAnne Tennant de Alonso, will be starting a post-doctoral position. He will continue to study the biology of overwintering monarchs and the Oyamel fir forest.

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Lincoln P. Brower, Chairman
Distinguished Service Professor
of Zoology

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Crawford S. Holling
Arthur R. Marshall, Jr., Professor of
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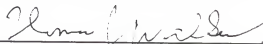
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This dissertation was submitted to the Graduate Faculty of the Department of Zoology in the College of Liberal Arts and Sciences and to the Graduate School and was accepted as partial fulfillment of the requirements for the degree of Doctor of Philosophy.

May 1996

Dean, Graduate School